THE 1987 FENITROTHION MONITORING STUDY ON SONGBIRD BRAIN CHOLINESTERASE ACTIVITY AND BALSAM FIR FOLIAGE

PREPARED FOR:

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EXECUTIVE SUMMARY

The Newfoundland and Labrador Department of Forest Resources and Lands (DFRL) conducted an aerial spray program to control an outbreak of the eastern hemlock looper (Lambdina fiscellaria fiscellaria) in insular Newfoundland during the summer of 1987. The primary insecticide used in the spray application was fenitrothion.

The DFRL was required as a condition of their Operator's Licence to undertake a monitoring study to assess the effect of fenitrothion on forest songbird populations.

The fenitrothion monitoring study area comprised 6 spray blocks located between Stephenville and Gros Morne National Park on the west coast of Newfoundland. The study area is within the Western and Central Newfoundland Ecoregions (Damman 1983). Much of the study area was cut over in the mid to late 1960's. Now the area is primarily composed of black spruce and balsam fir stands. Balsam fir foliage samples were collected to determine the amount of fenitrothion deposit reaching the forest vegetation.

The methodology chosen for the monitoring study was as follows:

- upper canopy foraging Blackpoll Warblers (Dendroica striata), mid canopy foraging Yellow-rumped Warblers (Dendroica coronata) and ground foraging White-throated Sparrows (Zonotrichia albicollis) were selected for the monitoring study
- forest songbirds were collected using .410 calibre shotguns
- songbirds were collected from unsprayed areas (control samples) for comparison to songbirds collected after fenitrothion spray applications
- songbird brains were removed and stored in liquid nitrogen for later analysis for cholinesterase (ChE) activity
- results were analyzed and compared with the literature
- foliage samples were collected in each spray block before and after spraying
- comparisons were made between foliage deposit values and songbird brain ChE activity

The mean brain ChE activity and its standard deviation were calculated for each species. Depressed activities for birds from sprayed areas were evaluated individually. It was necessary to establish criteria by which ChE activity could be judged normal

or depressed. The values calculated by Ludke et al. (1975) and Zinkl et al. (1980) were used in the data analysis to determine whether or not any particular songbird collected after spraying had a normal or depressed ChE activity. These values were the species control (i.e. unsprayed birds) sample mean less two standard deviations and the mean less 20% of the mean.

'Normal' birds would not likely have ChE activity below the above noted values, although in 1986, one control bird out of a sample of 56 control birds did (Fudge, Lane and Associates, 1987). If a songbird was collected from a sprayed area and its ChE activity was below the lower of these two values it was considered depressed due to the fenitrothion spray.

These values serve to indicate that there is some variability in ChE activity and to be certain of exposure to a ChE inhibiting agent the figures from the collected birds after spraying must be outside the normal amount of variation.

Field Results

The upper and mid canopy foraging warbler species showed a greater ChE depression after exposure to fenitrothion than did the ground foraging sparrow. In general, songbird brain ChE activity was depressed on the day of spray and further depressed on the day after spray when compared to control samples.

In one spray block songbirds were collected on the day of spray, first day after spray and the third day after spray following each of the two spray applications. In songbirds collected after the first spray there was evidence of a recovery in ChE levels on the third day after spray compared to the depressed levels on the day of spray and the day after spray collections. There was no evidence of recovery in ChE activity in songbirds sampled on the third day after spray following the second spray application to that block. Songbird sampling did not extend beyond day 3 and therefore the recovery of ChE activity beyond day 3 is unknown.

Approximately 50% of all the songbirds collected after spraying had ChE depressions indicating exposure to fenitrothion according to the criteria of Zinkl *et al*. (1980). Approximately 17% of all the

songbirds collected after spraying had ChE depressions indicating exposure to a potentially life-threatening amount of fenitrothion according to the criteria of Zinkl et al. (1980).

The songbirds (according to Zinkl et al. 1980) collected in this study that showed a potentially life-threatening depression in ChE activity were not evenly distributed amongst the 6 monitored spray blocks. Approximately 90% of those songbirds exhibiting a potentially life-threatening ChE depression were collected in 2 spray blocks.

There was a large variation in fenitrothion deposit in foliage samples within any particular spray block and large variation amongst spray blocks. The spray blocks that had the highest amount of fenitrothion deposit in the foliage samples did not yield the songbirds with the highest ChE depression.

Evidence suggests that in organophosphorus pesticides, the higher the dose of pesticide a bird ingests the greater the depression in ChE activity (Mineau and Peakall, 1987; Fleming and Bradbury, 1981; Fleming 1981; Fleming and Grue, 1981). In this study, although the amount of fenitrothion the songbirds are exposed to is not known, the vari-

ability in ChE activity results indicated that all songbirds did not ingest the same amount of the pesticide. The foliage deposit study indicates that the spray was not deposited uniformly on the forest vegetation and thus it may be reasonable to expect variations in ChE depression in songbirds following operational forest spray applications.

Nothing unusual or unexpected was seen, heard or otherwise detected in the behaviour of any bird species on the day of spray or days following spray. No songbird carcasses (other than the ones collected) were found in any block during this study.

Conclusions

The spraying of fenitrothion at 210 g AI/ha was shown to depress ChE activity in approximately 50% of the forest songbirds sampled. Seventeen percent of all songbirds collected exhibited a potentially life-threatening ChE depression following exposure to fenitrothion.

The results of the 1987 monitoring study indicate that there is reason for concern about forest songbird safety during operational forest spray activities using fenitrothion in Newfoundland.

Songbird brain ChE activity was monitored following both fenitrothion spray applications in one block. A trend toward a recovery in brain ChE activity was observed 3 days after the first spray applications. This trend indicating recovery in brain ChE activity was not observed up to 3 days after the second spray application on that spray block. The cumulative effect of the second spray application on songbirds that may have been previously exposed to fenitrothion is also an area of concern.

The typically mid and upper canopy foraging warbler species showed higher brain ChE depression than the typically ground foraging sparrow.

The depression in songbird brain ChE activity was not uniformly distributed in the spray blocks monitored. Approximately 90% of the songbirds that exhibited a potentially life-threatening depression in ChE following exposure to fenitrothion were collected in 2 spray blocks.

Monitoring studies such as brain ChE depression assess the effect of fenitrothion on individual songbirds. Field studies designed to assess fenitrothion effects at the population level are required.

A large amount of variation is observed in the fenitrothion deposit in foliage and songbird ChE depression in this monitoring study.

Recommendations

If fenitrothion is going to be applied in the future on forests in Newfoundland and Labrador, monitoring studies on songbirds should continue. Future monitoring studies should focus on the ability of songbirds to recover from fenitrothion after the first and second application.

The biological consequences of brain ChE depression are not resolved. Field studies aimed at determining how depressed ChE activity manifests itself in terms of breeding performance and overall fitness of affected songbirds are required. Effects monitoring studies at the population level are required to determine the biological consequences of operational fenitrothion spraying on forest songbirds in Newfoundland.

Foliage deposit studies should focus on collecting more samples throughout each block to determine a better average fenitrothion deposition for each block. Foliage samples should be collected for

several days following each spray to determine the length of time songbirds are capable of ingesting the spray (i.e. through preening and contact with foliage).

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1.0 INTRODUCTION

The Newfoundland and Labrador Department of Forest Resources and Lands (DFRL) aerially sprayed fenitrothion during the summers of 1985-1987 to control infestations of the eastern hemlock looper (Lambdina f. fiscellaria) in insular Newfoundland. In 1985 and 1986 DFRL was required as a condition of there Operators License to conduct a monitoring study on the effects of the spray application on songbirds.

Strong and Wells (1986) reported that between 29-100% of all individual songbirds collected from spray blocks had brain ChE activity less than 80% of average values for those species collected from control areas indicating probable exposure to fenitrothion during the 1985 monitoring study.

In 1986 Fudge, Lane and Associates Limited conducted a monitoring program during late July and early August in one operational spray block near Millertown. Brain cholinesterase (ChE) activity levels were found to be reduced in all songbird species and in 38% of the songbirds collected the ChE

depressions were considered to be potentially life-threatening (according to the criterion of Zinkl et al. 1980). On the basis of these preliminary results, it was recommended that DFRL review their spray formulation and application rate and that further and more extensive monitoring studies be conducted in the future (Fudge, Lane and Associates, 1987).

S. Fudge and Associates Limited was contracted to conduct the songbird monitoring study for the 1987 spray program in western Newfoundland. On the basis of recommendations given by a spray program review committee, a monitoring project of broader focus was conducted during the summer of 1987.

The eastern hemlock looper is a pest primarily restricted to insular Newfoundland. However, aerial spray programs against lepidopterous insect pests have been on-going in eastern Canada for decades.

The widespread and long-term use of insecticides for the control of the eastern spruce budworm (Choristoneura fumiferana) in eastern Canada has been

reviewed by Mineau and Peakall (1984). In eastern Canada, eastern spruce budworm control operations have resulted in organochlorines, organophosphates and carbamates being broadcast over large areas of balsam fir (Abies balsamea) and spruce (Picea spp.) forests (Mineau and Peakall 1984, Peakall and Bart 1983).

Fenitrothion (O, O - dimethyl - O - <3 - methyl - 4-nitrophenyl > phosphorothioate) has been the major insecticide used to control the eastern spruce budworm since 1968 (Busby et al. 1983). Fenitrothion, an organophosphate, is a ChE inhibitor (Silver 1975).

ChE inhibitors have been used increasingly as insecticides since the initiation of restrictions on chlorinated hydrocarbons (Zinkl et al. 1979). ChE inhibitors block synaptic transmissions in the cholinergic parts of the nervous system by binding to the active site of acetylcholinesterase, which normally hydrolyses the neurotransmitter acetylcholine to choline and acetic acid. The ChE inhibitors thus permit excessive acetylcholine accumu-

lation at synapses and disrupt or block nerve impulse transfer (Corbett 1974). Acetylcholinesterase inhibition probably causes death in air-breathing vertebrates by blocking neurotransmissions in the respiratory center of the brain or at myoneural junctions of respiratory muscles (O'Brien 1967, Corbett 1974). Mortality of songbirds has been reported from fenitrothion use in forest insect control programs (Grue et al. 1983).

Fenitrothion is applied aerially to the forest canopy thereby exposing many non-target organisms, including songbirds to the toxin (Busby et al. 1983).

Avian response to insecticide spraying has traditionally been monitored by comparing pre- and post-spray singing activity of territorial males, with a decrease in singing activity being assumed to indicate a detrimental effect (Pearce et al. 1976, 1979; Busby et al. 1983). Singing bird surveys are confounded by uneven spray application, replacement of birds killed (Stewart and Aldrich 1951, Hensley and Cope 1951), adverse weather conditions and bird migration (Pearce et al. 1976, Peakall and Bart

1983). Signs of ChE inhibitor poisoning in birds include myosis, salivation, lacrimation (muscurinic effects), muscle twitching, dyspnoea, paralysis and clonic convulsions (nicotinic effects) (Zinkl et al. 1977). In addition, the fate of most birds in areas of reduced singing activity remains unknown (Busby et al. 1983).

Since brain ChE activity is easily measured, its measurement can be used to determine if a bird has been poisoned with organophosphate or carbamate insecticides (Stickel 1974). Brain ChE measures an adverse physiological response (Busby et al. 1983). However, the ChE activity of birds suspected to have been sprayed with ChE inhibitors must be compared with that of unsprayed birds of the same species because of the variation in ChE activity between species (Stickel 1974).

Brain ChE determination is becoming widely accepted as a method of assessing exposure of birds to ChE inhibiting insecticides (Mineau and Peakall, 1987; Fudge, Lane and Associates 1987; Busby et al. 1981,

1983; Strong and Wells 1986; White et al. 1979; Zinkl et al. 1979, 1981).

In 1987, balsam fir foliage was collected in each of the monitored spray blocks to determine if there was any relationship between the amount of fenitrothion residue on the foliage and the amount of brain ChE depression observed in forest songbirds collected in the blocks.

1.1 OBJECTIVES

The objective of this study was to assess the effects on forest songbirds of the 1987 DFRL fenitrothion spray application to control eastern hemlock looper in western Newfoundland.

1.2 STUDY AREA

Six operational forest spray blocks were monitored in this study. Five spray blocks were situated between Stephenville and Rocky Harbour on the west coast in the Western Newfoundland Ecoregion (Damman 1983). One

spray block was situated in the Central Newfoundland Ecoregion (Damman 1983).

The Western Newfoundland Ecoregion is characterized by balsam fir (Abies balsamea). Black spruce (Picea mariana) stands are present but primarily restricted to poorly drained sites and bedrock outcrops. Mountain maple (Acer spicatum) thickets are abundant and seem to replace the speckled alder (Alnus rugosa) of the Central Newfoundland Ecoregion in moist nutrient rich sites.

The Central Newfoundland Ecoregion is characterized by balsam fir and black spruce forests. Stands of poplar (Populus tremuloides) and white birch (Betula papyrifera) are common in the study area. Scrub areas of poplar and alder and regenerating cut-over areas also occur in the study area. Dwarf shrub heath dominated by Kalmia angustifolia and small areas of Sphagnum spp. bog can occur locally.

Some of the spray blocks have been logged since the mid to late 1960's. The regenerated areas are composed primarily of immature (0-20 years) balsam

fir and black spruce under 3.5 m in height. Mature stands of balsam fir and black spruce (40-80 years) 9.6 - 12.5 m in height are common in the spray blocks that were monitored. The location of the monitored spray blocks is shown in Figure 1.1.

1.3 MATERIALS AND METHODS

1.3.1 Spray Application Details

The technical details of the spray application and spray mixture are provided in Tables 1.1 and 1.2.

1.3.2 Sampling Design

Of the nine spray blocks (#'s 101-109) that were designated by DFRL to be sprayed by DC-6 aircraft, four were selected based on access for songbird collections (Figure 1.1). Preliminary reconnaissance determined that each block had good road access and sufficient area to support enough of the targeted songbird species for sampling. Spray block 103, the largest block in the area, was divided into three smaller spray blocks (103a - Lines 21-35, 103b-

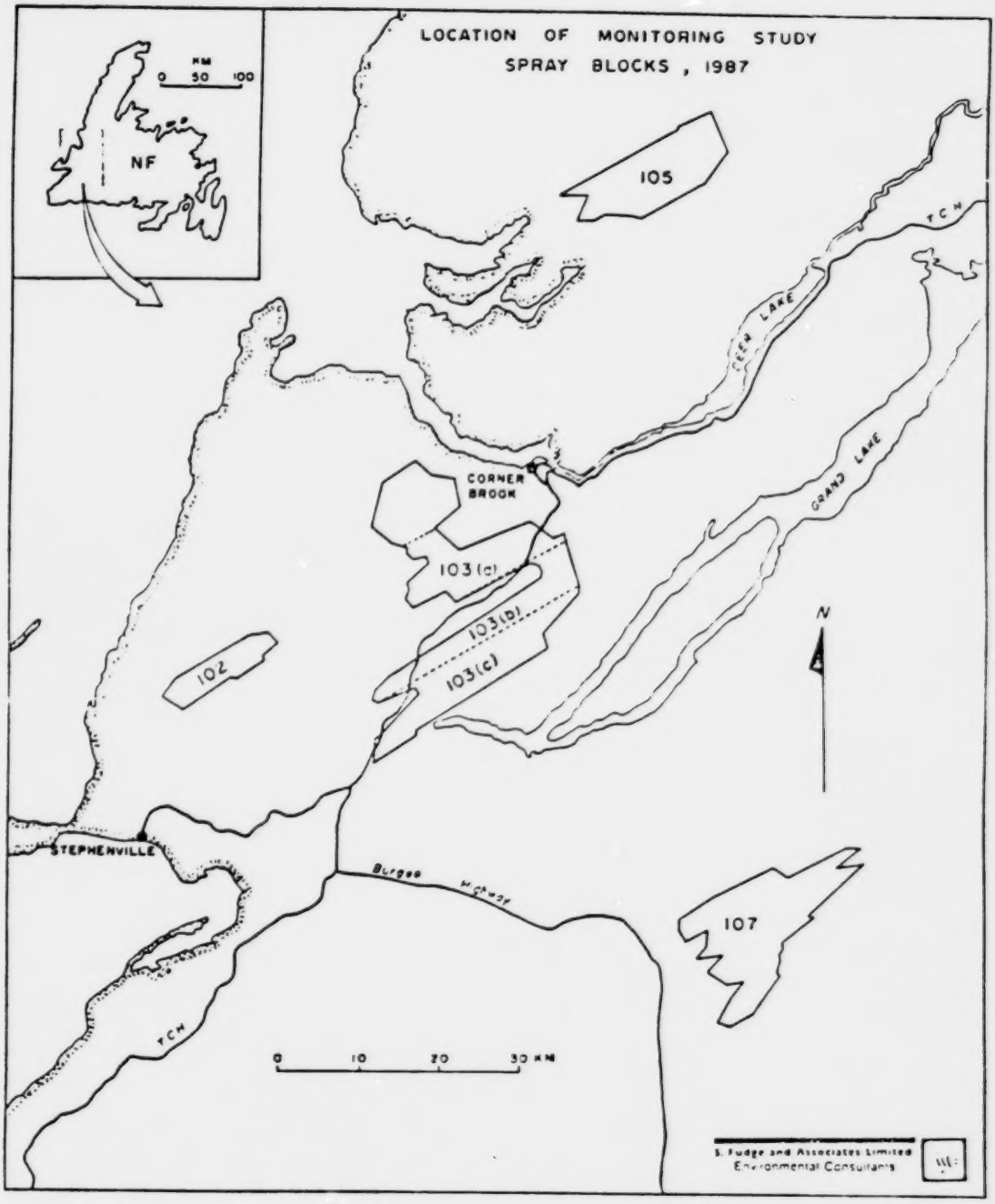


FIGURE 1.1 : SPRAY BLOCK LOCATIONS

Table 1.1 Technical details of spray application and formulation for the 1987 hemlock looper control program.

Spray Application

Aircraft: DC-6
Aircraft height: 61 m
Aircraft Speed: 305 km/h
Spray Apparatus: Boom with 200 nozzles with #8010 spray tip
Swath Width: 610 m

Spray Mixture

Insecticide:	fenitrothion (Folithion^{R})
Mixture by Volume:	11% fenitrothion
	40% Cyclosol 63
	49% ID 585 (Insecticide Diluent)

Application Rate

210 g active ingredient per 1.5L per ha in 2 applications

Table 1.2 Spray dates and sampling schedule for the 1987 fenitrothion spray monitoring project.

BLOCK	SPRAY DATE	START FINISH	COLLECTIONS DAY 0	DAY 1	DAY 3
102	87-07-13	2116 - 2145 h	87-07-14	87-07-15	-
103a	87-07-12	0556 - 0700 h	87-07-12	87-07-13	-
103b	87-07-17	0530 - 0650 h	87-07-17	87-07-18	-
103c	87-07-03	0605 - 0705 h	87-07-03	87-07-04	-
107	87-07-18	0550 - 0645 h	87-07-18	87-07-19	-
105 1st	87-07-09	0550 - 0657 h	87-07-09	87-07-10	87-07-12
105 2nd	87-07-20	2045 - 2140 h	87-07-21	87-07-22	87-07-24

Lines 10-20 and 103c - Lines 1-9), each of which was sprayed separately thereby resulting in a total of six operational spray blocks.

Six spray blocks were chosen for the monitoring study in 1987 to determine if variation exists in spray deposit amongst blocks and whether songbirds collected from different spray blocks exhibit similar or variable ChE depression.

Two sampling designs were established as per the Terms of Reference for this study (NDOE). In five of the six spray blocks (#'s 102, 103a, 103b, 103c and 107), songbirds were collected on the day of the spray application (Day 0) and during the following day (Day 1) for the first application only. These spray blocks were referred to as the extensive blocks. In the remaining spray block (#105), songbirds were collected on Day 0, Day 1 and on Day 3 (the third day after Day 0) for the first and second spray applications. This block was referred to as the intensive block. If a block was sprayed during the evening, the following day was considered to be Day 0.

Five extensive blocks were sampled to assess the effect of the 1st spray application on songbirds on the day of spray and first day following spray. One intensive block was sampled to look at the effects of 2 operational sprays in the same areas songbirds and more days following sprays were monitored to see if any observed ChE depression would recover.

.3.3 Songbird Collections

Two species of warbler and one species of sparrow were collected. These species were chosen because they were believed to be relatively abundant in the study area and would provide a representative cross-section of songbirds inhabiting lower, middle and upper forest canopy. The White-throated Sparrow (Zonotrichia albicollis) is generally a low canopy or ground level forager and is common in open, immature forest stands. The Yellow-rumped Warbler (Dendroica coronata) typically forages in the mid to upper canopy in mature forests. The Blackpoll Warbler (Dendroica striata) occurs in a variety of forest types, but generally forages in the upper canopy.

Brain ChE activity is species specific therefore control samples of each species must be collected for comparison to individual songbirds collected after exposure to fenitrothion. Songbird species were selected on the basis of their typical foraging level in the forest. It is assumed that upper canopy foragers should be exposed to more fenitrothion spray than mid-canopy forages. And it follows that mid-canopy foragers should be exposed to more fenitrothion spray than ground foraging songbirds.

However, in large spray blocks in western Newfoundland there are often large areas of forest regeneration and areas of open 'Christmas tree' growth. In these areas typically ground foraging songbirds commonly forage at tree top level and typically mid and upper canopy forages are found within a very shallow forest vegetation profile.

Recovery of depressions in bird brain ChE activity due to exposure to organophosphorus pesticides have been reported (Fleming 1981; Fleming and Bradbury, 1981; Fleming and Grue, 1981). It was assumed that by collecting songbirds for several days after

exposure to fenitrothion a similar recovery in brain ChE could be observed.

A ten person (six songbird study, four foliage study) field crew was established in the study area on 23 June 1987. Control songbirds of each species were collected in unsprayed areas, in or near the spray blocks, during a four day period (26 - 29 June) prior to the day the first extensive block was sprayed.

For the extensive spray blocks, attempts were made to collect ten specimens each of Blackpoll Warblers and White-throated Sparrows on Day 0 and on Day 1. In block 107 it soon became evident that it would not be possible to obtain enough Blackpoll Warblers, thus Yellow-rumped Warblers were collected instead.

In the intensive block, attempts were made to collect ten specimens of all three species on each of Day 0, Day 1 and Day 3, after both the first and second spray applications. The dates and times of spraying and songbird collections are presented for each spray block in Table 1.2.

Songbirds were collected by shooting using .410 calibre shotguns with #6, #7 1/2 or #9 lead shot. Collections began no sooner than 1 h after the spray had been completed. In general, only adult male birds were collected. Males were usually located by song, then "pished in" or "whistled in" to obtain a clear shot at close range. Hunting was avoided in areas near the spray boundaries and designated shut-off areas within the blocks to reduce the chance of collecting unexposed birds that flew into the sprayed area after spraying had ceased.

Songbird collectors attempted to collect the daily quota of songbirds as soon in the day as possible. If a block was sprayed in the evening the collectors were in the field the next morning at daybreak trying to collect songbirds.

The collection of songbirds in any particular spray block was not pre-determined or conducted in a 'random' basis. The distribution of songbird collecting effort was influenced first by road access in the spray blocks and second by suitable habitat in accessible areas. There are certain risks encoun-

tered in a field program where access is restricted and songbird collecting is confined to several small areas. If problems were encountered in the spray delivery (e.g. accidental shut-offs, heavy deliveries, double swaths) in those areas, the songbirds collected may not provide a representative sample. However, during the 1987 spray project no abnormal spray effects were reported to the field crews that would make songbird collections suspect in terms of data interpretation.

After shooting, specimens were taken to an area where a liquid nitrogen container was located. Brains were removed from the specimens, placed in serum vials and stored in liquid nitrogen until time of assays. Generally, specimens were processed within 1 h of collecting and never longer than 2 h. For each specimen, the percentage of brain that remained after collection was recorded. Brains which had received heavy shot damage were not used in the analysis because different areas of the brain are reported to have different ChE activities (Knittle and Tucker 1974, from Zinkl et al. 1977). Statistical analysis was conducted on data from whole (> 95% total brain) brains collected from adult male specimens. This was

necessary to avoid any possible differences in ChE activity due to sex or age (immature vs. mature) or damage to brain samples.

Brain ChE assays were conducted by the Canadian Wildlife Service, Fredericton, New Brunswick and followed the colourimetric techniques of Ellman et al. (1961) as adapted by Hill and Fleming (1982).

Whole brain samples were used in the hydrolysis techniques and the final reported ChE value was an average of 2 assays on each brain sample. Prior to assaying, all samples were randomized to prevent possible bias from processing sequential groups of specimens under laboratory conditions which can vary slightly from day to day. Brain tissue analysis was completed on 10 August 1987.

Individual songbird brain ChE activity is given in Appendix A.

As an adjunct to the information derived from songbird collections, field notes were kept by the sampling crew to provide a record of songbird

behaviour and other activities during the breeding season. A minor criticism of the 1986 spray monitoring study (Fudge, Lane and Associates 1987) was that fewer songbirds were collected after the spray than before the spray. The low collecting success may be because collections started late in the summer and certain species had finished breeding and migrated from the area. To avoid this potential problem of data interpretation during the 1987 study, the field crew attempted to document the phenology of the breeding activities for each songbird species through field observations.

1.3.4 Foliage Collections

Samples of balsam fir foliage were collected in each of the monitored spray blocks after each spray event. Thus each of the extensive blocks had foliage samples collected after the first spray application and the intensive block had foliage samples collected after each of the two spray applications.

Control balsam fir foliage samples were collected in spray blocks prior to spraying to compare them with

the foliage samples collected in the spray blocks after spraying.

In each spray block, 5 transects, 1 km in length, situated perpendicular to the spray application flight lines were established. The transect locations were determined on a near random basis depending on access and no two transects were situated closer than 160 m.

On each of the 5 transects in each of the study blocks, 5 balsam fir trees were selected for sampling on a near random basis. From each of the balsam fir trees approximately 30 g of new growth needles was collected from all sides of the mid-canopy region, approximately at the 4 cardinal compass directions. The foliage samples from the 5 trees were then combined to yield one bulk foliage sample for each transect. Each foliage sample was collected carefully ensuring the foliage was not touched by the collectors. Bulk samples were placed in opaque plastic bags and stored in a freezer prior to shipping to the laboratory for analysis.

Control foliage was collected in unsprayed areas before each spray block was sprayed. In each control area 25 foliage samples were collected and grouped into 5 large samples.

The laboratory determination of fenitrothion residue in treatment and control samples was based on Reynolds (1977) and performed by the Environmental Protection Service, St. John's. Methodology for fenitrothion determination in foliage samples is provided in Appendix B.

1.3.5 Data Analysis

Birds collected in each block on the different collection days were considered to be individual samples. The data on the level of brain ChE activity from the chemical analysis were sorted according to day of collection within each block for each species. These data were tested for normality and homogeneity of variance. Differences among blocks and days of collection were analyzed for each species using non-parametric tests when the data was skewed or heterogenous, otherwise parametric tests were used.

The level of rejection for all statistical tests was $p=0.05$.

These data were also viewed according to the criterion of Zinkl *et al*. (1980). The standard, threshold ChE activity below which songbirds are considered to have been exposed to a ChE inhibiting agent is the lower of two values, either $\bar{X} - .2\bar{X}$ or $\bar{X}$ -2 SD (standard deviation) of the control mean (Zinkl *et al*. 1980). A 50% inhibition of brain cholinesterase ($\bar{X} - .5\bar{X}$) is considered to be life-threatening. Because of the acknowledged sampling bias that is expected to result from collecting mainly "healthy" birds and to facilitate inter-study comparisons (Mineau and Peakall 1984), the data were also presented as proportions of individuals in the sample that exhibit these three levels of ChE reduction.

The results of the balsam fir foliage deposit analysis were compared with the results of the songbird brain ChE analysis to determine if there was any trend between the two.

1.4 STUDY TEAM PERSONNEL

Stephen M. Fudge	- Project Manager
David Lemon	- Project co-ordinator and report preparation
Malcolm Macartney	- Field supervisor, data analysis
Margaret Purdy	- Field supervisor, data analysis
Bruce Mactavish	- Field Supervisor
Sandra Meron	- Field Supervisor
Pierre Ryan	- Field Biologist
David Robbins	- Field Biologist
Perry Trimper	- Field Biologist
David Pitcher	- Field Biologist
Wade Thomas	- Field Biologist
Gerard English	- Field Biologist
Perry Diamond	- Data analysis, field biologist and drafting

1.5 ACKNOWLEDGEMENTS

S. Fudge and Associates Limited would like to acknowledge the assistance provided by Corner Brook

Pulp and Paper Limited, the Department of Forest Resources and Lands, the Department of Environment and Fisheries and Oceans Canada.

Mr. D. Busby and Mr. P. Pearce of the Canadian Wildlife Service provided helpful information for the report preparation and analyzed the songbird brain samples for ChE activity.

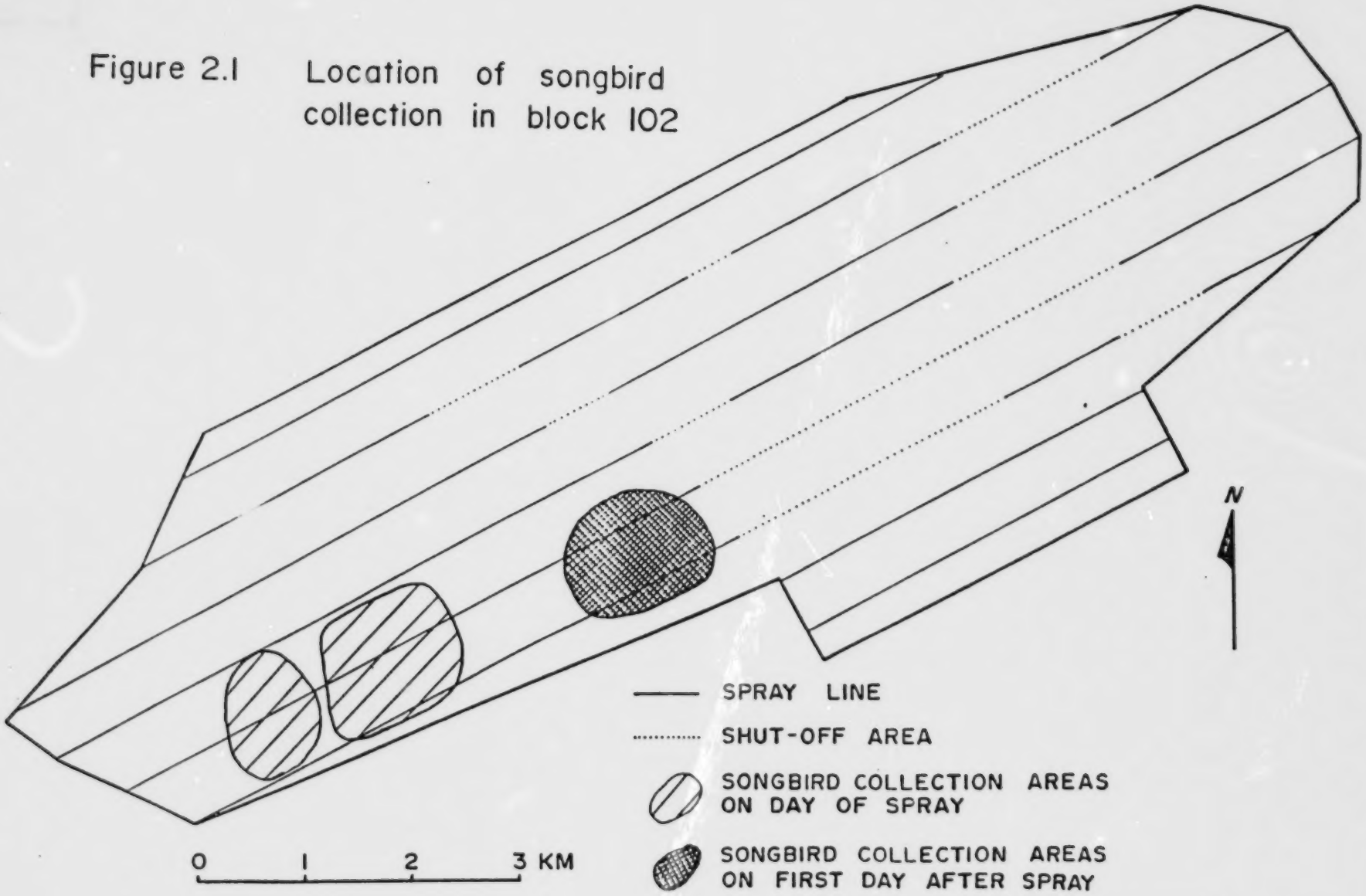
2.0 RESULTS

The location of songbirds in each spray block are shown in Figures 2.1, 2.2, 2.3, 2.4, 2.5, 2.6, and 2.7.

2.1 COMPARISON OF SONGBIRDS COLLECTED IN EXTENSIVE SPRAY BLOCKS

A comparison of all extensive spray blocks and the first spray application to the intensive spray block (day of spray, first day after spray) was made for Blackpoll Warblers and White-throated Sparrows.

Figure 2.1 Location of songbird collection in block 102


N
SPRAY LINE
SHUT-OFF AREA
SONGBIRD COLLECTION AREAS ON DAY OF SPRAY
SONGBIRD COLLECTION AREAS ON FIRST DAY AFTER SPRAY
0 1 2 3 KM

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Environmental Consultants

Figure 2.2 Location of songbird collection in block 103(a)

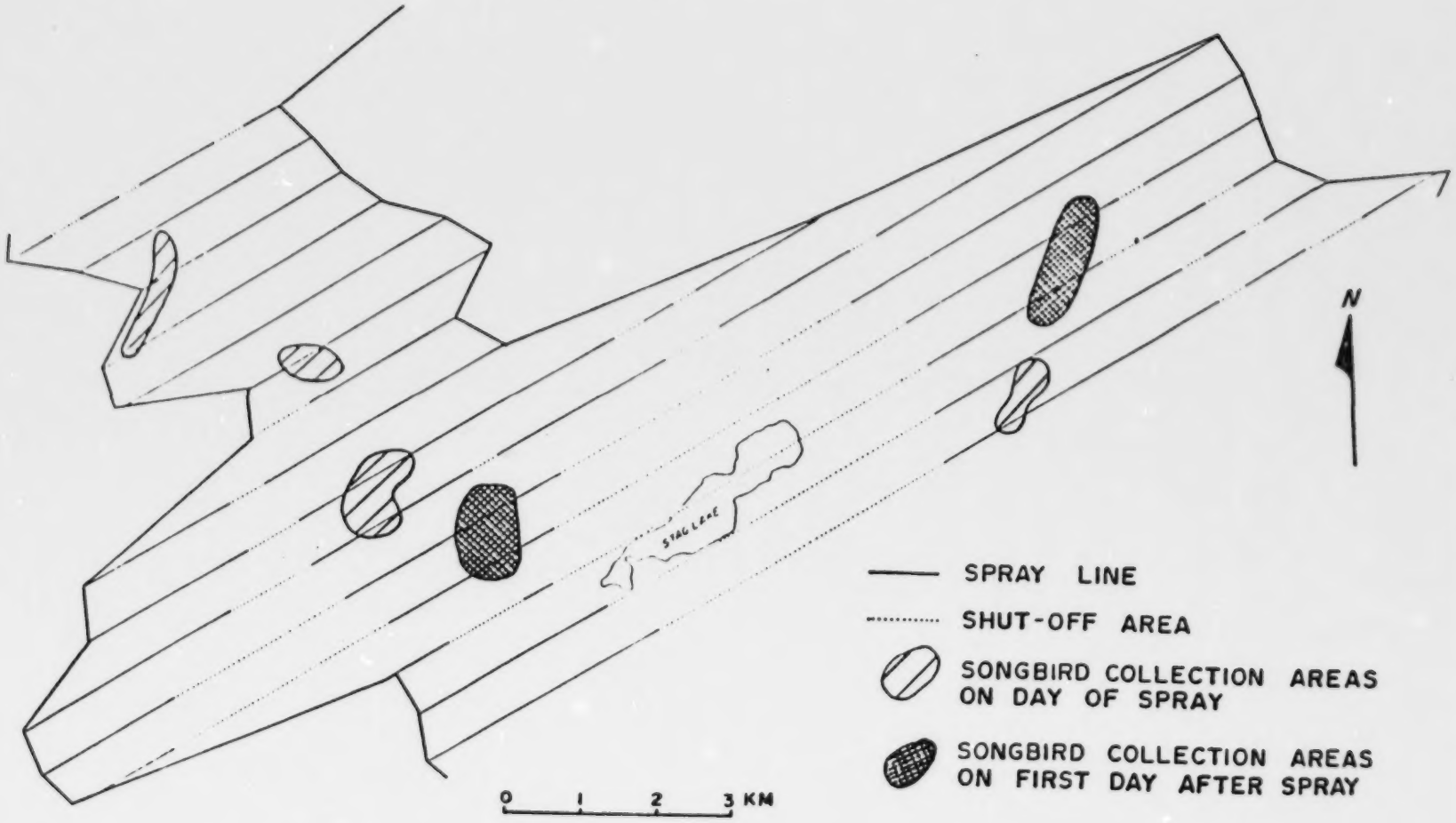

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Figure 2.3 Location of songbird collection in block 103(b)

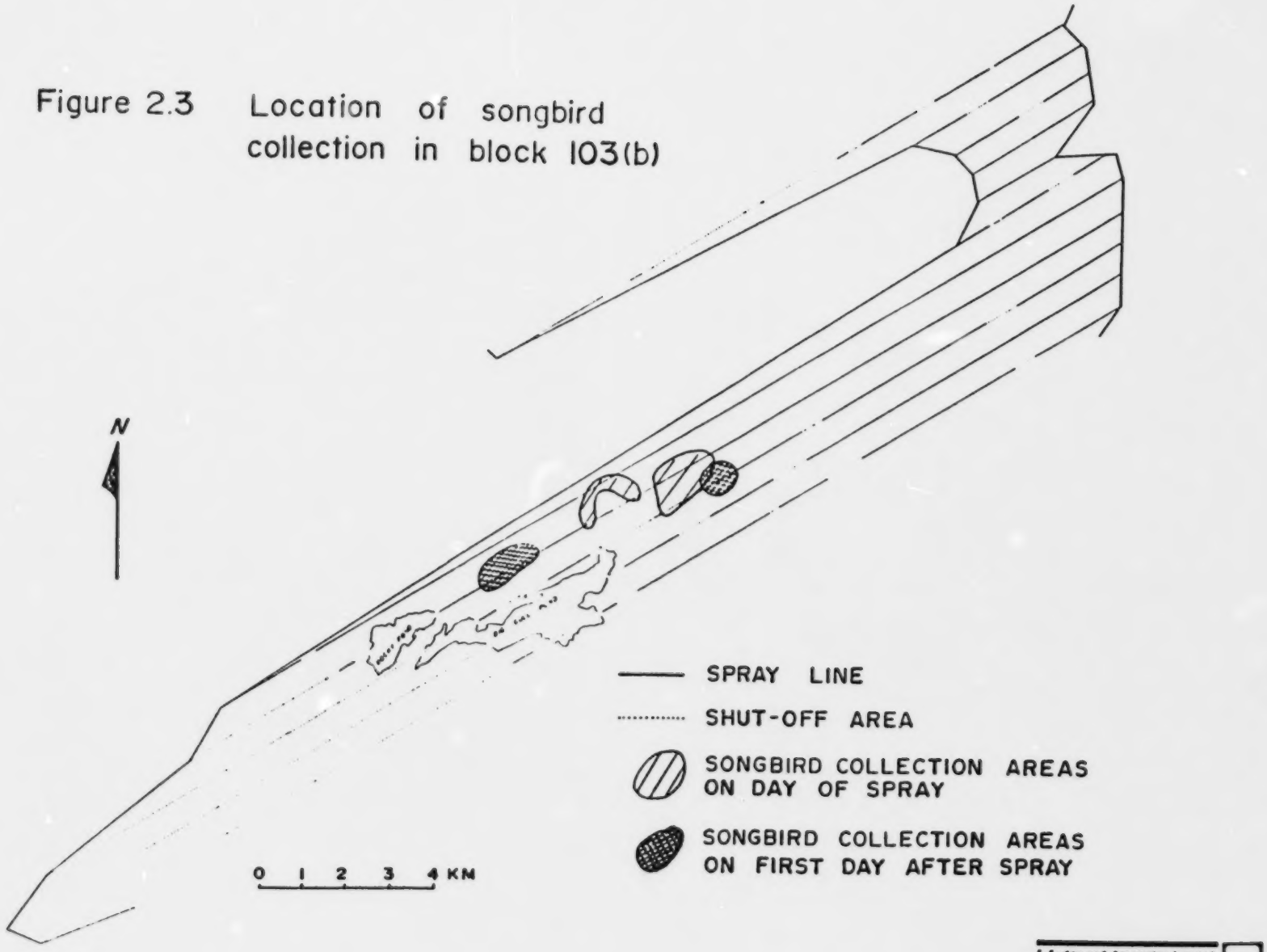

N
0 1 2 3 4 KM
SPRAY LINE
SHUT-OFF AREA
SONGBIRD COLLECTION AREAS ON DAY OF SPRAY
SONGBIRD COLLECTION AREAS ON FIRST DAY AFTER SPRAY
S. Fudge and Associates Limited
Environmental Consultants

Figure 2.4 Location of songbird collection in block 103(c)

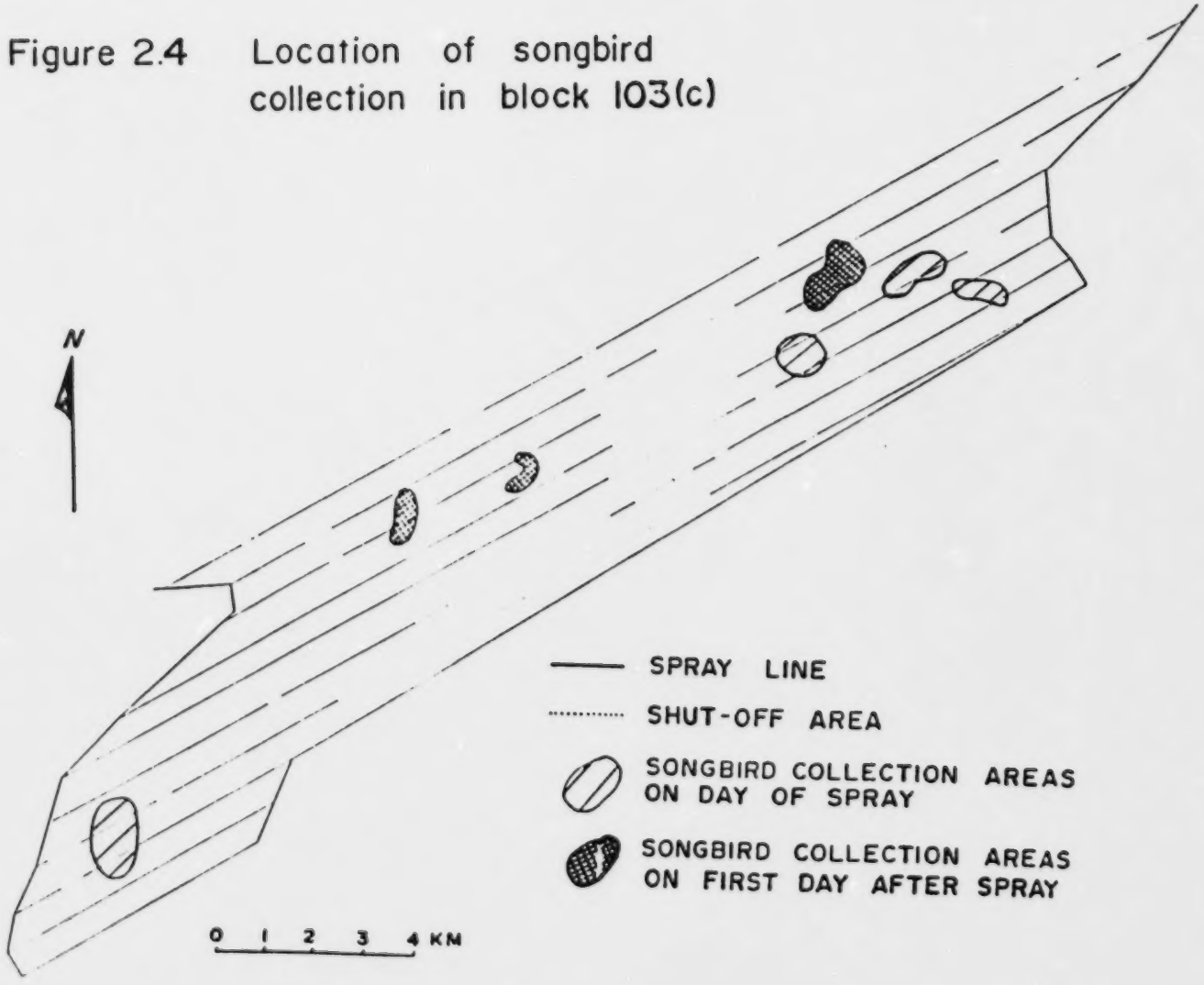

N
SPRAY LINE
SHUT-OFF AREA
SONGBIRD COLLECTION AREAS ON DAY OF SPRAY
SONGBIRD COLLECTION AREAS ON FIRST DAY AFTER SPRAY
0 1 2 3 4 KM

Figure 2.5 Location of songbird collection in block 105 (first application)

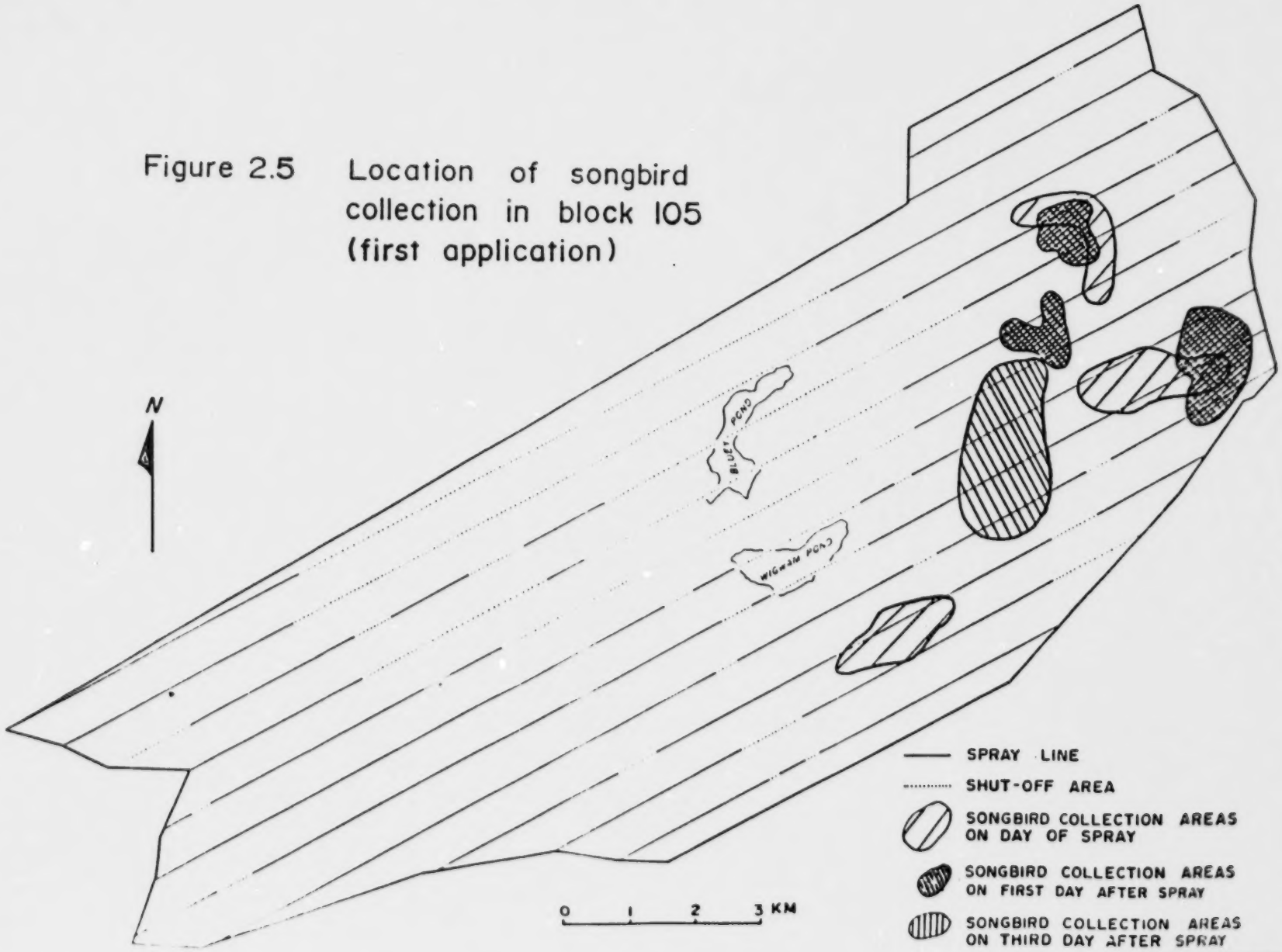

N
BUSY POND
WIGWAM POND
0 1 2 3 KM
SPRAY LINE
SHUT-OFF AREA
SONGBIRD COLLECTION AREAS ON DAY OF SPRAY
SONGBIRD COLLECTION AREAS ON FIRST DAY AFTER SPRAY
SONGBIRD COLLECTION AREAS ON THIRD DAY AFTER SPRAY

Figure 2.6 Location of songbird collection in block 105 (second application)

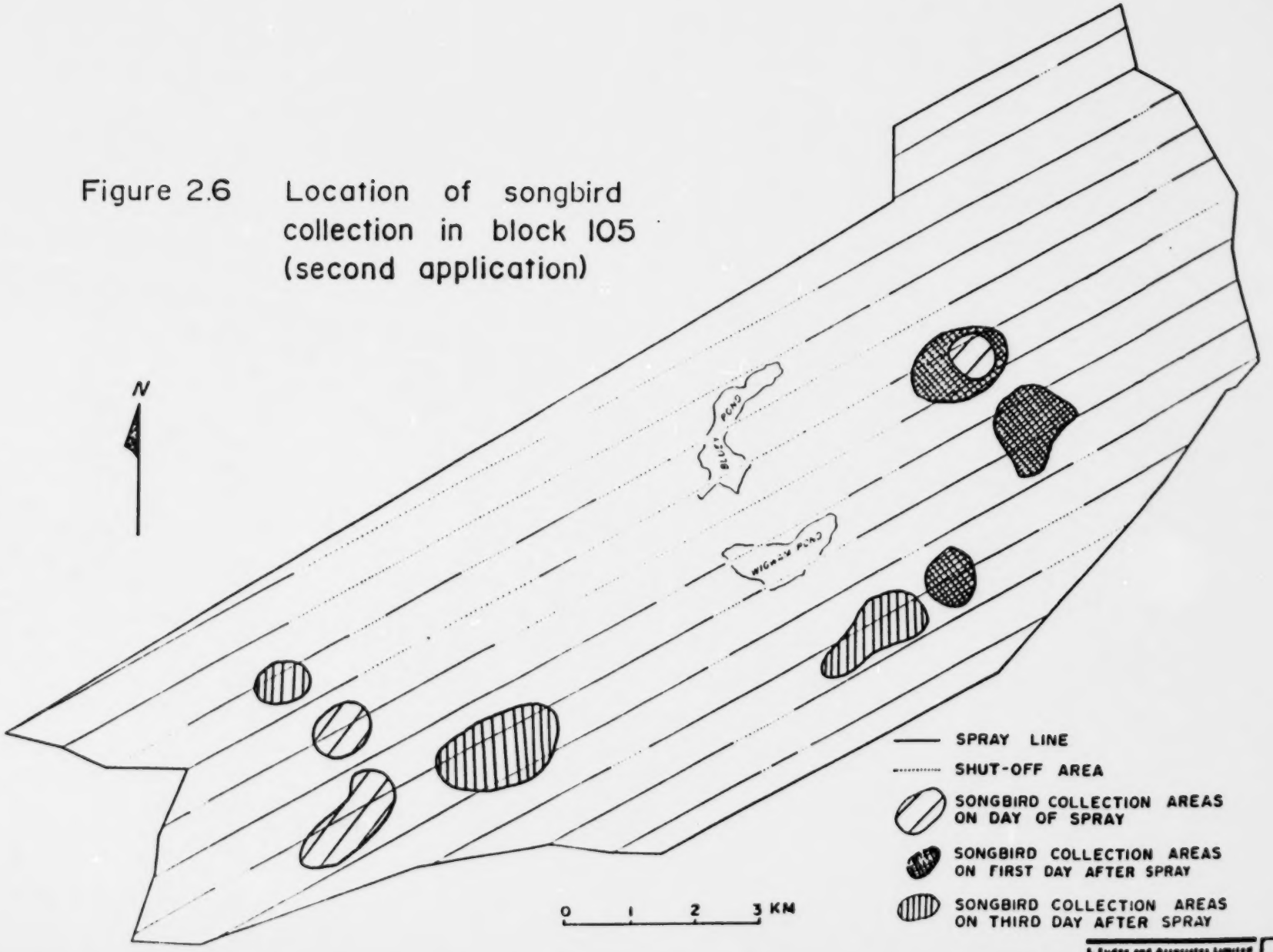
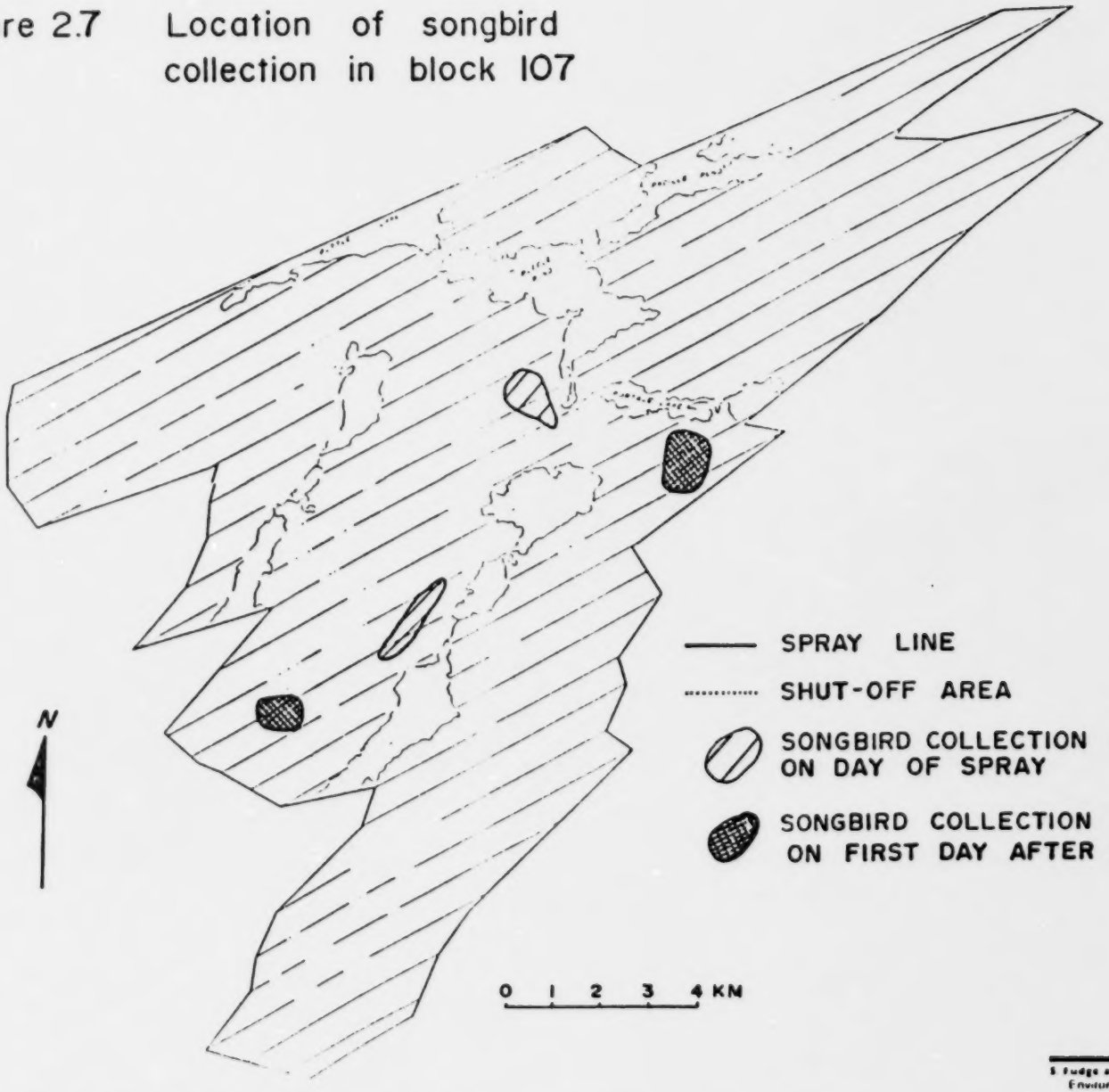

N
BLUFF POND
HIGHWAY POND
0 1 2 3 KM
SPRAY LINE
SHUT-OFF AREA
SONGBIRD COLLECTION AREAS ON DAY OF SPRAY
SONGBIRD COLLECTION AREAS ON FIRST DAY AFTER SPRAY
SONGBIRD COLLECTION AREAS ON THIRD DAY AFTER SPRAY

Figure 2.7 Location of songbird collection in block 107


SPRAY LINE
SHUT-OFF AREA
SONGBIRD COLLECTION AREAS ON DAY OF SPRAY
SONGBIRD COLLECTION AREAS ON FIRST DAY AFTER SPRAY
N
0 1 2 3 4 KM

The sample size (n), and sample mean ($\bar{X}$) for Blackpoll Warbler samples are shown in Figure 2.8.

The n and $\bar{X}$ for the White-throated Sparrow samples are shown in Figure 2.9.

2.2 COMPARISON OF SONGBIRDS COLLECTED IN INTENSIVE SPRAY BLOCK

A comparison of the songbirds collected in the intensive spray block was made for Blackpoll Warblers, Yellow-rumped Warblers and White-throated Sparrows.

The n and $\bar{X}$ for Blackpoll Warblers are shown in Figure 2.10.

The n and $\bar{X}$ for Yellow-rumped Warblers are shown in Figure 2.11.

The n and $\bar{X}$ for White-throated Sparrows are shown in Figure 2.12.

2.3 OBSERVATIONS OF SONGBIRD ACTIVITIES

When the collecting crew arrived in the field during the last week of June songbirds were singing at high

Figure 2.8 Brain ChE activity in Blackpoll Warblers from different spray blocks. Figure indicates the mean, and the range (vertical line) with the sample size indicated for each block.
(_____ day of spray; - - - day after spray)
* According to the criterion of Zinkl et al. 1980.

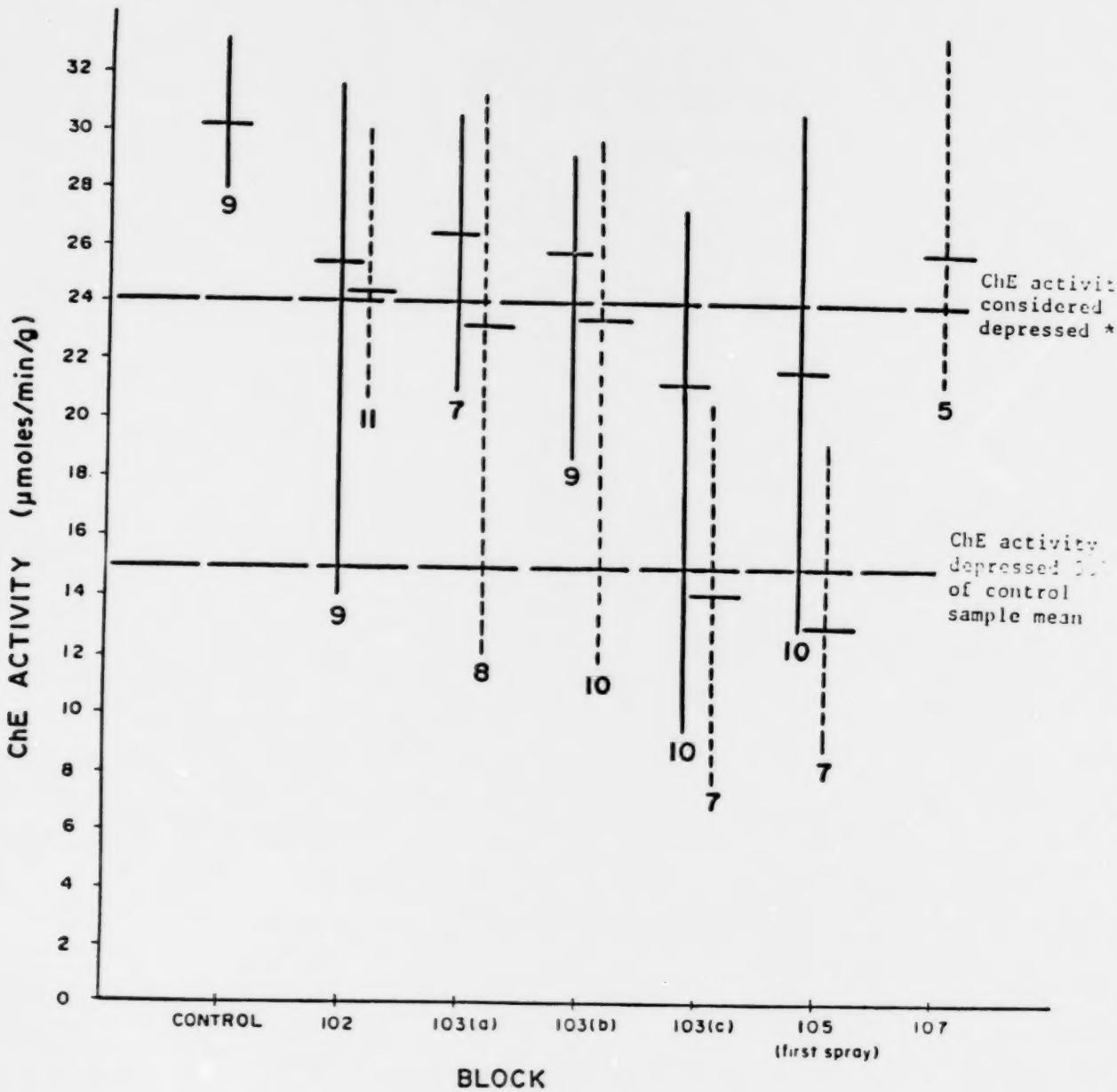


Figure 2.9 Brain ChE activity in White-throated Sparrows from different spray blocks. Figure indicates the mean, and the range (vertical line) with the sample size indicated for each block. (_____ day of spray; - - - day after spray)

* According to the criterion of Zinkl et al. 1980.

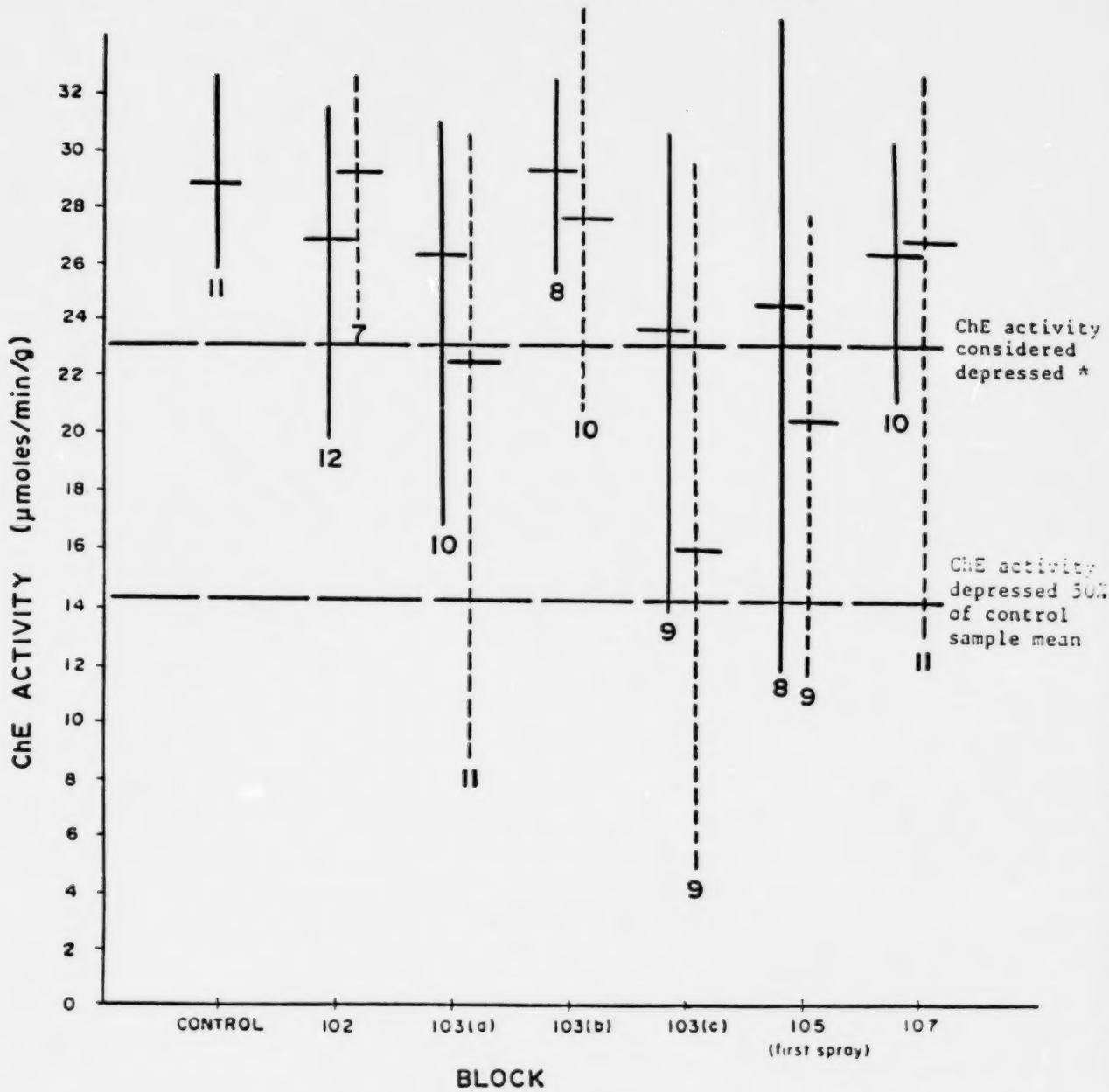


Figure 2.10 Brain ChE activity in Blackpoll Warblers from block 105 after first and second spray applications. Figure indicates the mean, and the range (vertical line) with sample size indicated for each day.
* According to the criterion of Zinkl et al. 1980.

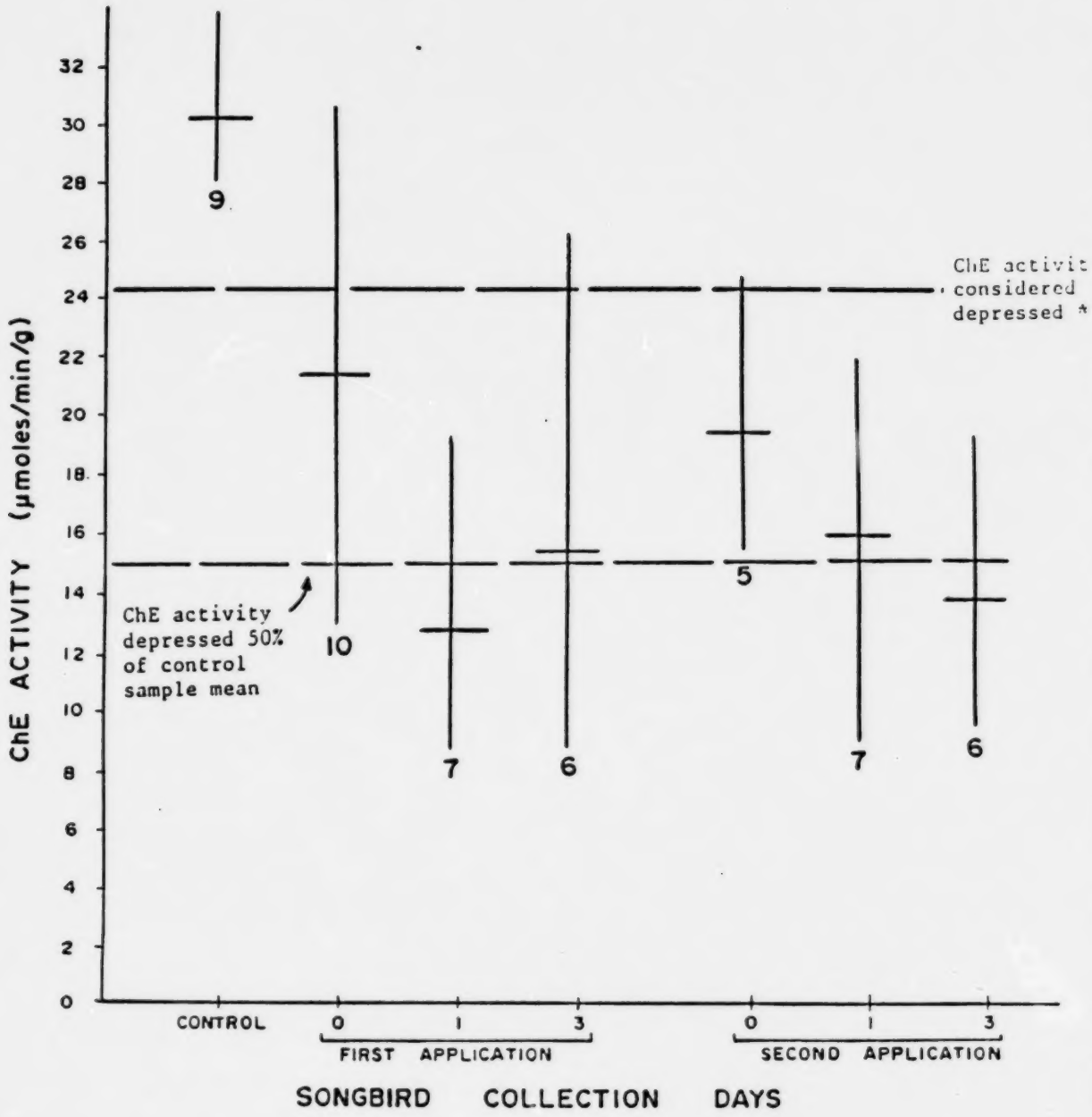


Figure 2.11 Brain ChE activity in Yellow-rumped Warblers from block 105 after first and second spray applications. Figure indicates the mean, and the range (vertical line) with sample size indicated for each day.

* According to the criterion of Zinkl et al. 1980.

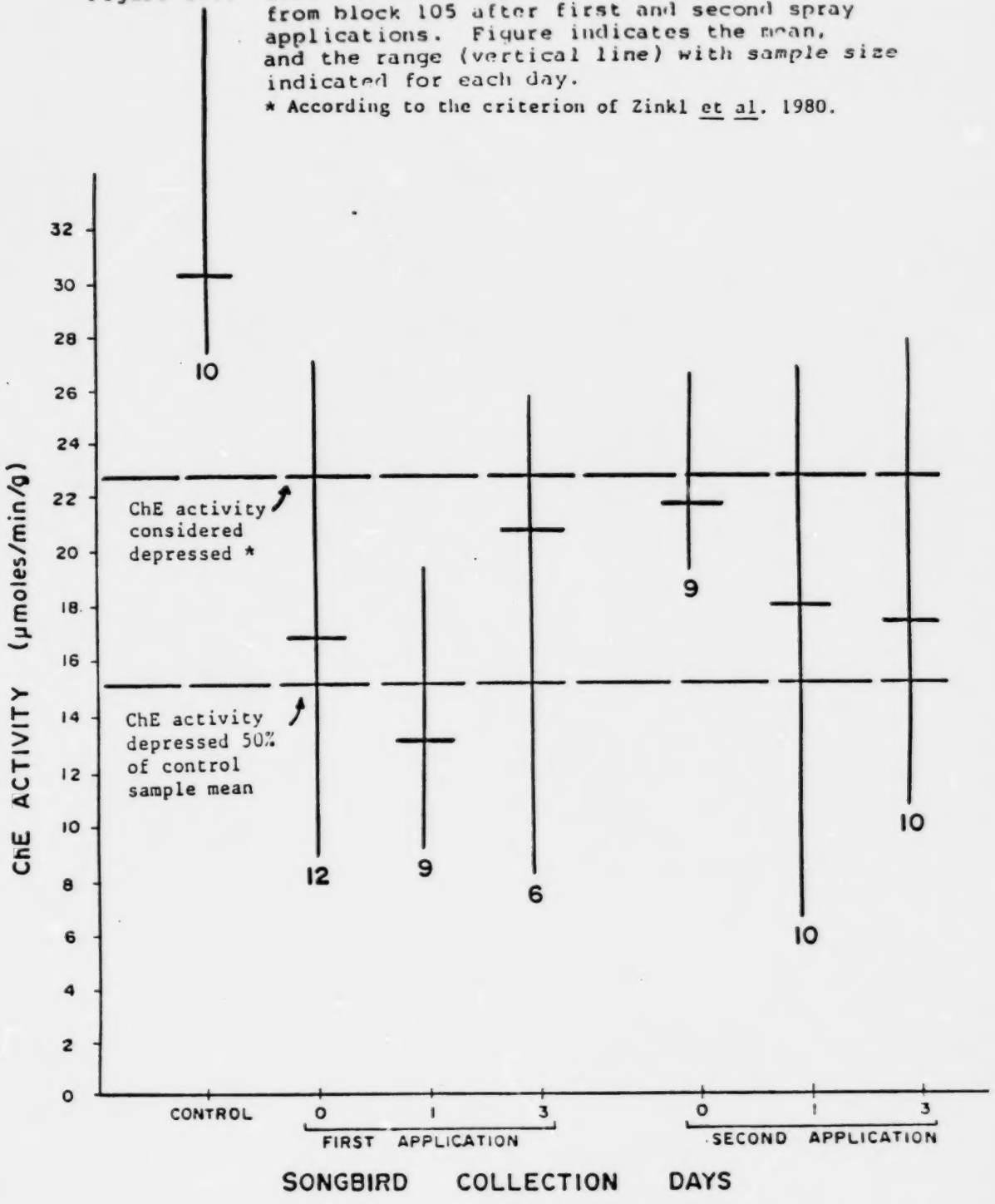
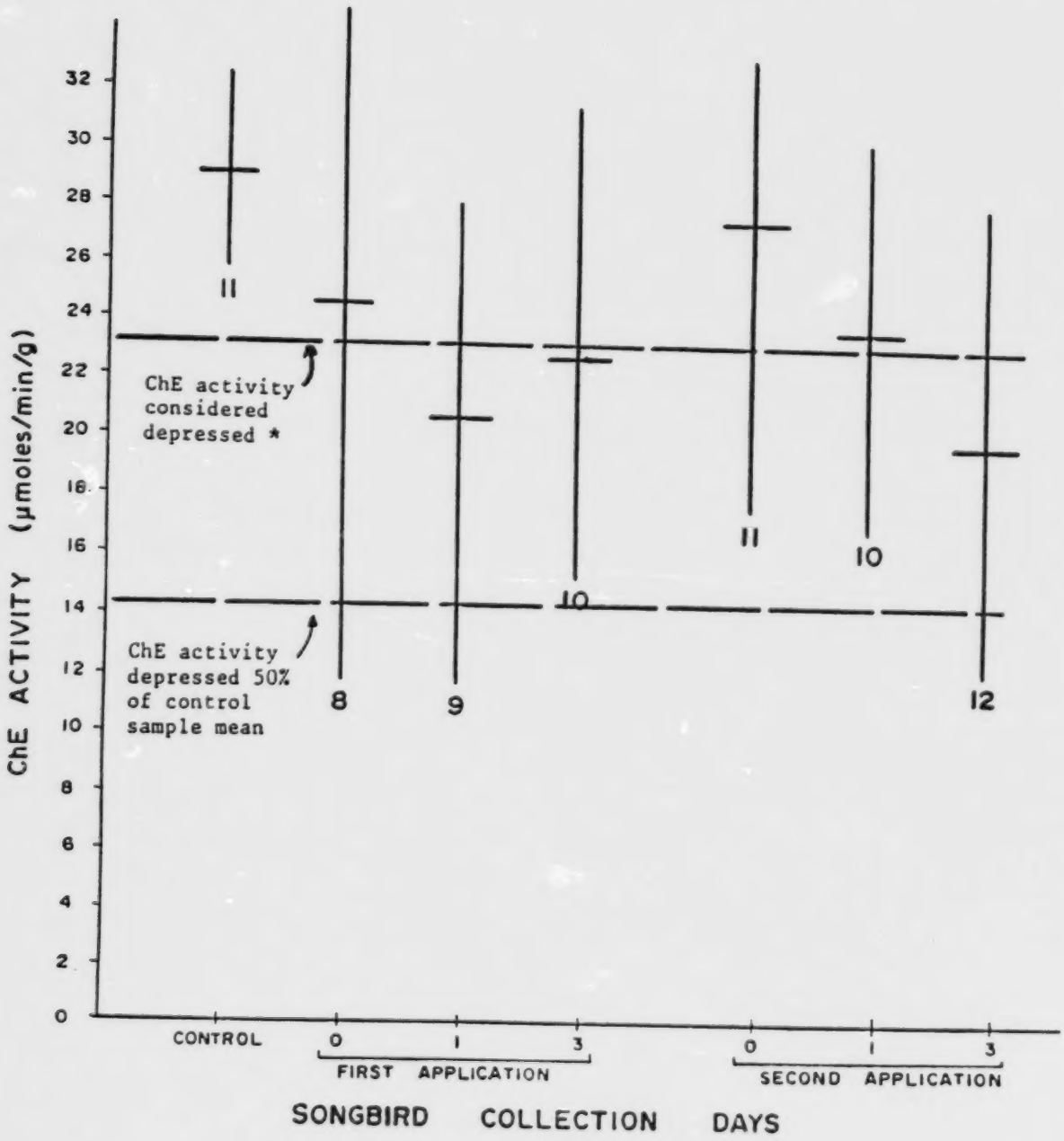


Figure 2.12 Brain ChE activity in White-throated Sparrows from block 105 after first and second spray applications. Figure indicates the mean, and the range (vertical line) with sample size indicated for each day.

* According to the criterion of Zinkl et al. 1980.



levels for the breeding period. Typically a decrease in volume and length of singing after dawn was observed for White-throated Sparrows and American Robins (Turdus migratorius) during the first week of July. Similarly, warblers, thrushes and other forest species were all singing with less vigor in early July. This reduction in singing coincided with the appearance of the first young birds observed in the field. On 4 July, broods of newly fledged White-throated Sparrows were noted by crews in two different parts of Block 103.

By the second week of July singing had become more sporadic by most species and occurred on a regular basis only on clear, calm mornings. Most of the days in July were unusually warm and by late morning birds sang only intermittently or stopped singing altogether. The forest in both unsprayed and sprayed areas was extremely quiet on days when conditions were less than ideal i.e. when it was too windy, too hot or too humid. Fledged family groups of White-throated Sparrows and Lincoln's Sparrows (Melospiza lincolni) were frequently seen at this time. On 10 July the first family group of Yellow-rumped Warblers

was observed in a spray block. After 12 July, male Yellow-rumped Warblers were seldom heard singing, but family groups became more numerous in areas of suitable habitat.

After 15 July there was a decrease in the amount of singing by male Blackpoll Warblers, and females started to respond to "pishing" more readily than males. This was probably because females were off the nest and both sexes were involved in feeding chicks. On 17 July a Blackpoll Warbler nest containing 4 young (pin feathers on wing) was located. The same day a family group of Blackpoll Warblers (three young) with both parents feeding was observed.

From mid-July until the end of the collections, the calls of young birds of various species were frequently heard in the woods. Adult birds, especially males, responded less and less to "pishing" and imitated calls, probably because most of young were out of the nest and the urge to guard territory was not as strong. Blackpoll Warblers became very hard to locate.

Collections in Block 107 on 18 and 19 July were hampered by strong winds and lack of suitable habitat for Blackpoll Warblers. The forest stands in this higher elevation area were largely tall, mature spruce and open spruce bog. Blackpoll Warblers were rare, Yellow-rumped Warblers were common and some males were still singing in this block, thus it was decided to switch target species from Blackpoll Warblers to Yellow-rumped Warblers.

Sampling for the second spray application in the intensive block 105 began on the clear, calm morning of 21 July. Yellow-rumped Warblers sang in the early morning, and White-throated Sparrows and Blackpoll Warblers sang in bursts. Only five Blackpoll Warblers were collected on this day because sampling started in the western end of the block which was mainly mature black spruce woods, poor habitat for Blackpoll Warblers. Five specimens were eventually shot in the eastern portion of the block during the hot sunny afternoon. Had collections started in the eastern portion of block 105, more Blackpoll Warblers may have been collected.

On 22 July the woods were very quiet. Most observations of Yellow-rumped Warblers were of females travelling and feeding fledglings (but unaccompanied by males). Juvenile Yellow-rumped Warblers flying on their own were also observed. Most of the male Blackpoll Warblers collected on 22 July were moulting as indicated by the beginnings of post nuptial fall plumage coming in along the sides of the breast and top of the head.

On the final day of collecting singing had become very sporadic in the species still singing; Mourning Warbler (Oporornis philadelphia), Blackpoll Warbler and White-throated Sparrow. Many female Yellow-rumped Warblers, males and family groups were observed. Most fledgling White-throated Sparrows were fully feathered and flying. The Blackpoll Warblers were very secretive and had to be stalked.

The general consensus from all songbird collectors was that nothing unusual or unexpected was seen, heard or otherwise detected in the behaviour of any bird species on the day of spray or days following

spray. No songbird carcasses (other than the ones collected) were found in any block during this study.

2.4 FENITROTHION DEPOSIT IN BALSAM FIR FOLIAGE

The location of foliage collection transects in each of the spray blocks is shown in Figures 2.13, 2.14, 2.15, 2.16, 2.17, and 2.18. The results of the foliage analysis are presented in Table 2.1.

3.0 DATA ANALYSIS

Songbird samples were tested for differences among blocks and collection days for each species.

3.1 DIFFERENCES AMONG BLOCKS - EXTENSIVE BLOCKS

3.1.1 Blackpoll Warbler - Day of Spray

Differences between all spray blocks on the day of spray was tested for Blackpoll Warbler samples including the control samples. There was a significant difference among sample means (ANOVA

Figure 2.13 Location of foliage collection transects in block 102

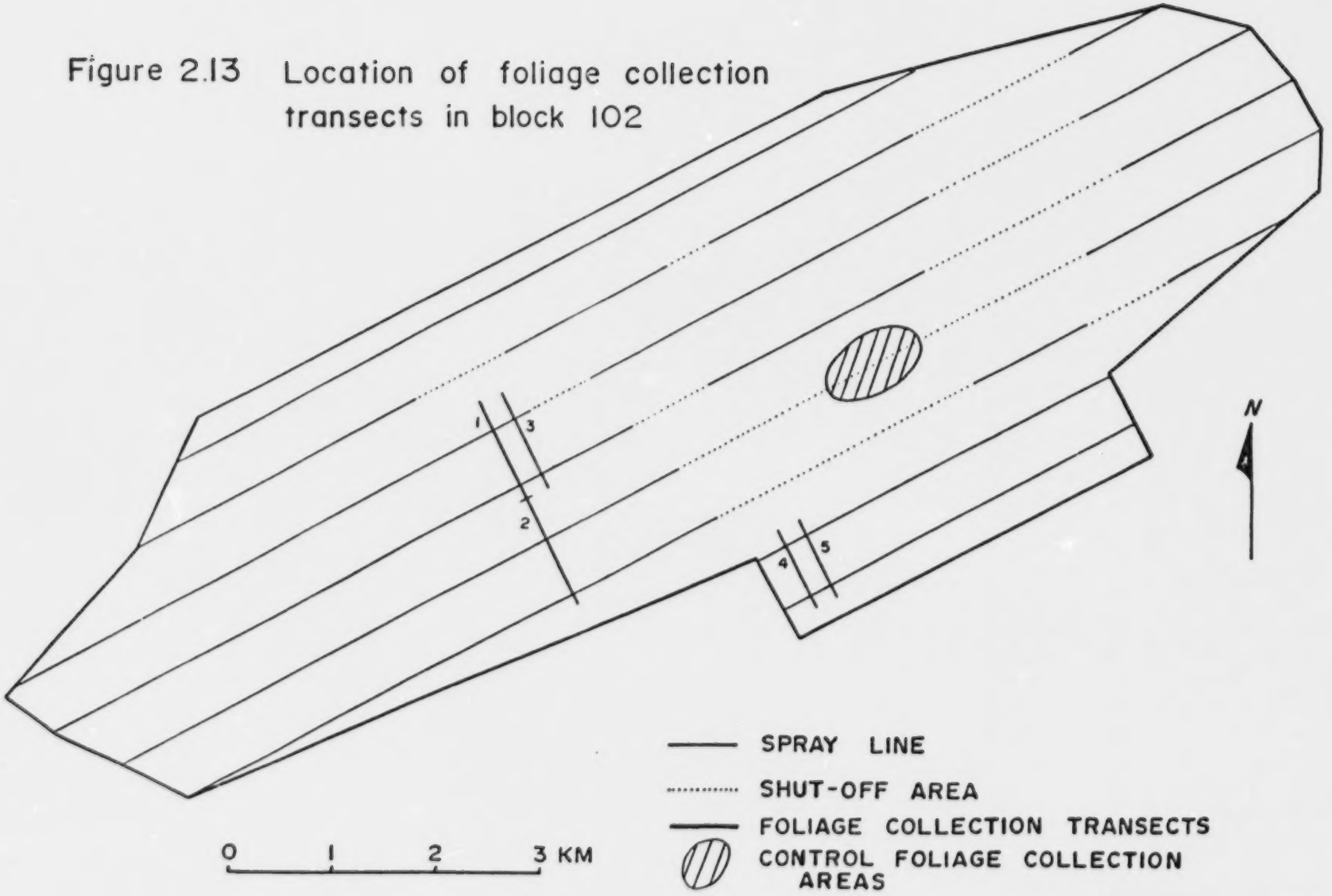


Figure 2.14 Location of foliage collection transects in block 103(a)

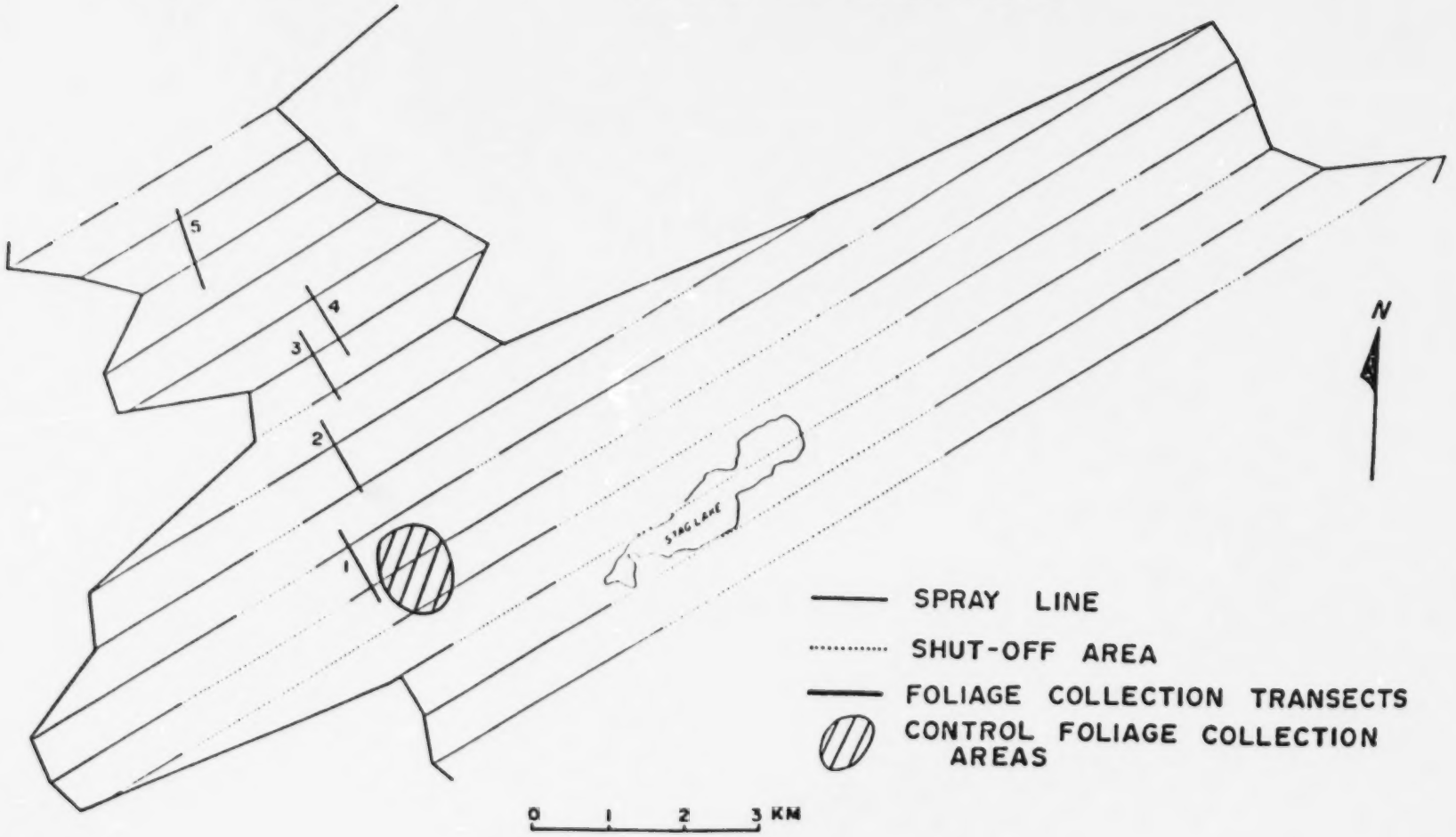


Figure 2.15 Location of foliage collection transects in block 103(b)

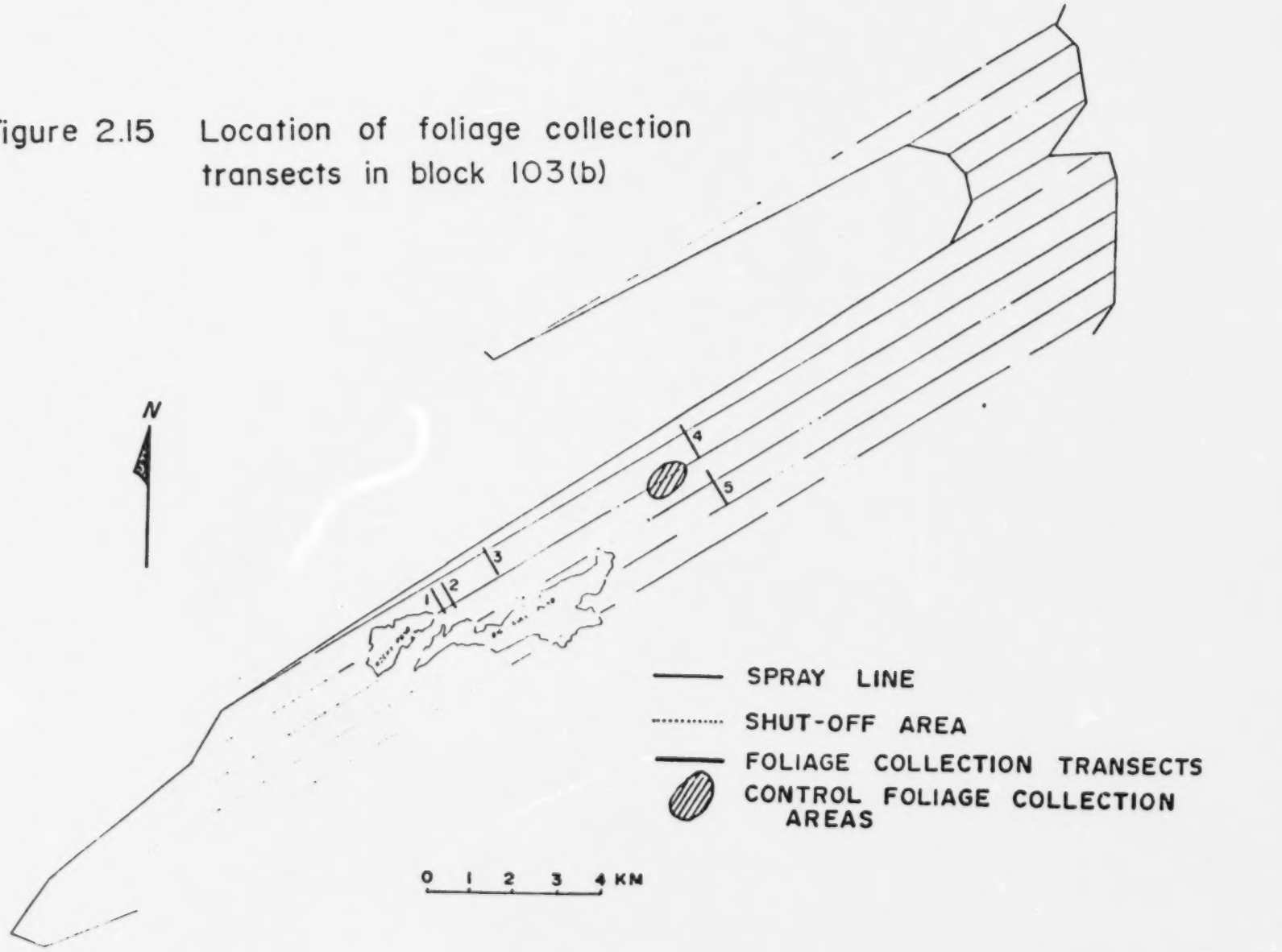


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Figure 2.16 Location of foliage collection transects in block 103(c)

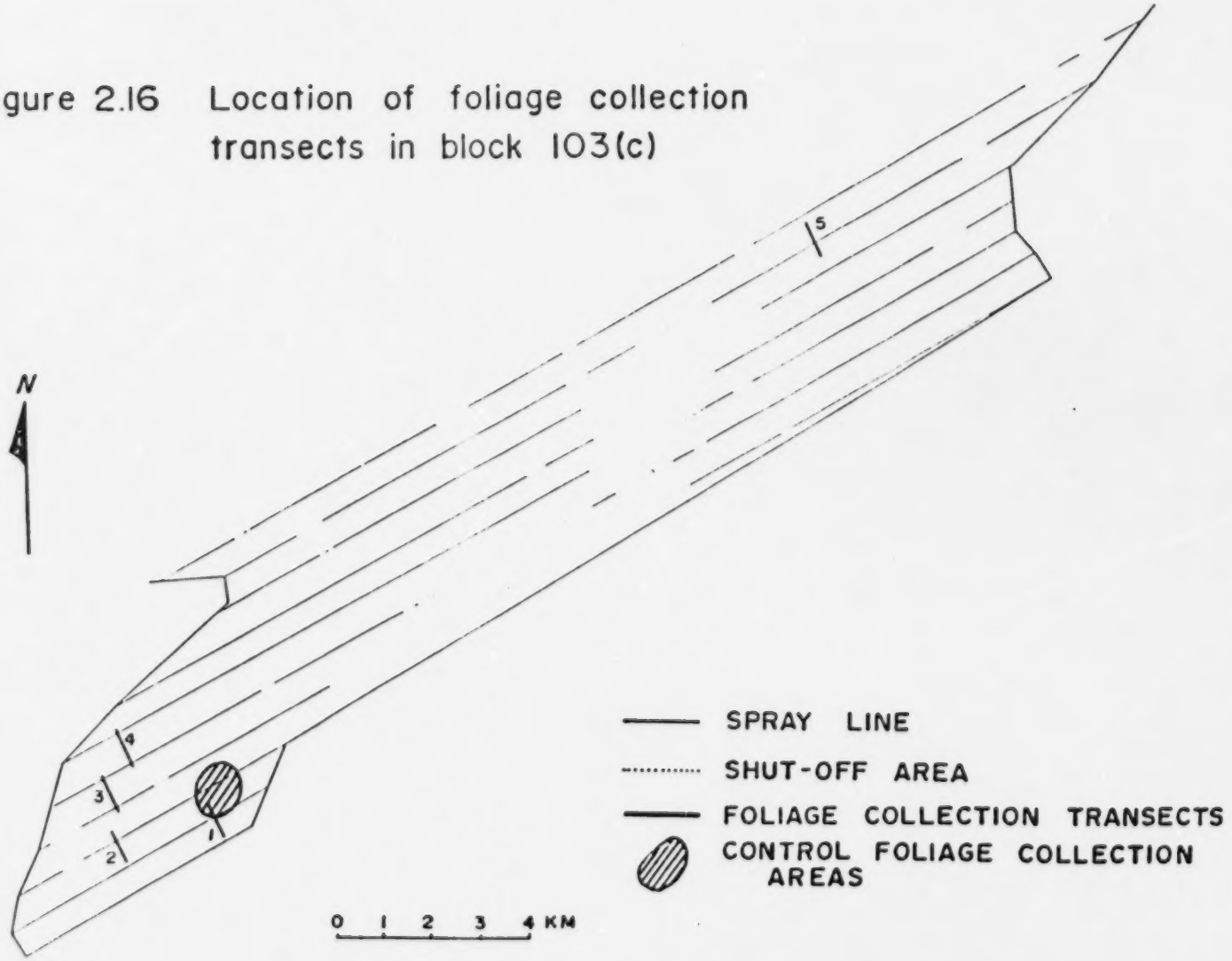

Figure 2.17 Location of foliage collection transects in block 105 (first and second applications)

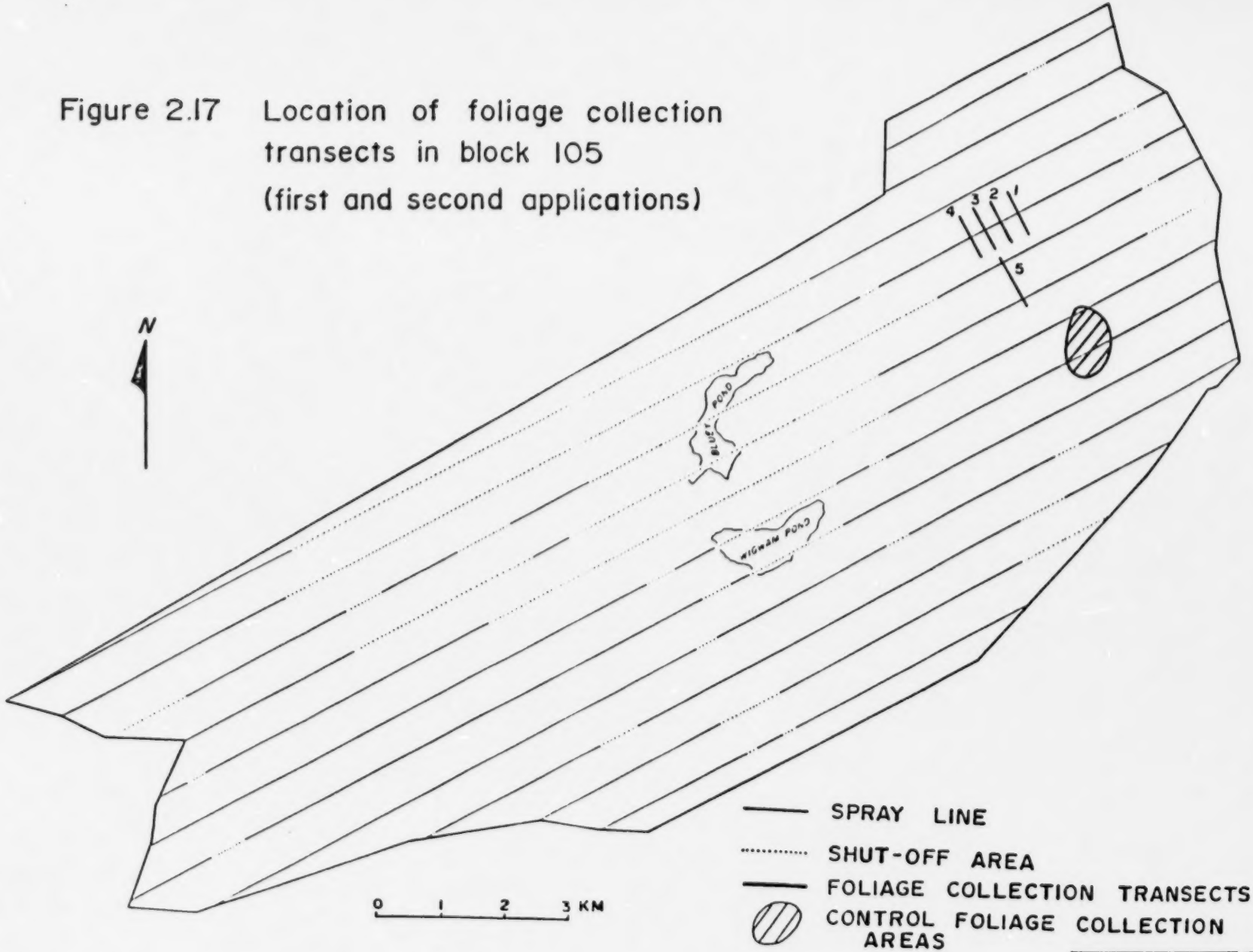

Figure 2.18 Location of foliage collection transects in block 107

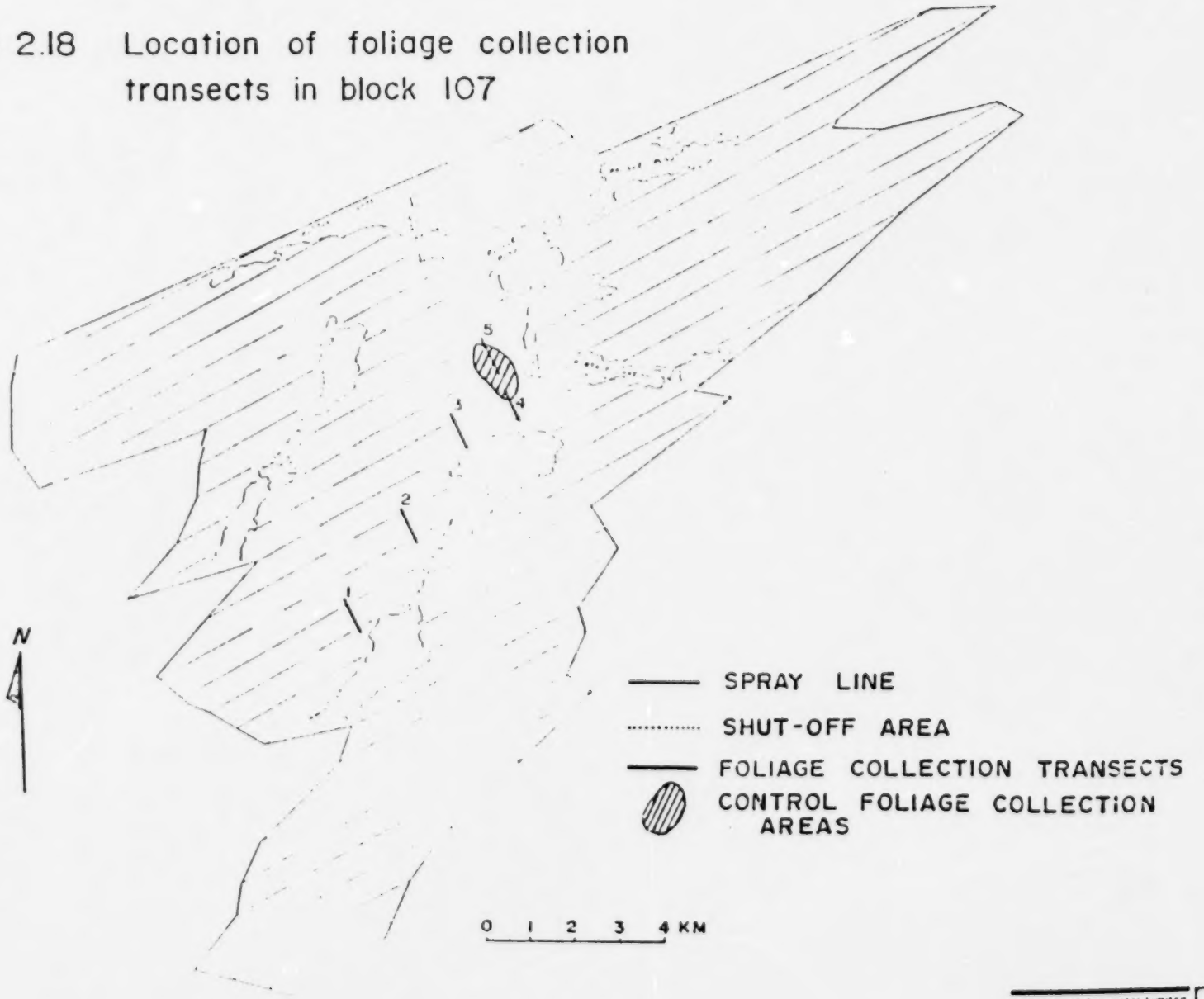

Table 2.1 Results of the 1987 fenitrothion deposit analysis in balsam fir foliage collected in each of the spray blocks where songbirds were collected. Values expressed as µg/g.

Block number	Sample # 1	2	3	4	5	Sample Mean for block
102 - control	0.04	0.03	0.02	0.03	0.03	0.03
- spray	13.50	1.54	2.97	3.55	2.92	4.90
103(a)-control	0.02	0.01	<0.01	<0.01	0.02	0.01
-spray	8.04	5.17	6.11	1.64	2.07	4.60
103(b)-control	0.02	0.02	<0.01	<0.01	<0.01	0.01
-spray	1.46	0.56	1.46	1.20	0.92	1.12
103(c)-control	<0.01	0.01	<0.01	<0.01	<0.01	0.00
-spray	0.93	<0.01	3.90	3.25	4.30	2.48
105 - control	<0.01	0.02	0.01	<0.01	<0.01	0.01
- 1st spray	0.73	1.70	0.58	1.60	3.90	1.70
- 2nd spray	1.88	1.65	2.80	1.45	3.58	1.44
107 - control	<0.01	<0.01	<0.01	<0.01	<0.01	0.00
- spray	0.48	0.75	0.23	0.58	1.70	0.75

F=4.48, d.f. = 5, n = 6. P < .01). A subsequent multiple range test (Duncan's) was inconclusive. However, blocks 105 and 103c showed the greatest ChE depression and were significantly different from the control samples (Duncan's, Newman-Keuls multiple range test). Blocks 102, 103b and 103a were moderately depressed compared to the control samples (Figure 2.8).

3.1.2 Blackpoll Warbler - Day After Spray

There was a significant difference (ANOVA F=13.58, d.f.= 5, n = 7, P < .01) between sample population means on the day after spray. A subsequent multiple range test (Duncan's and Newman-Keuls multiple range test) identified significant differences between control and blocks 105/103c; and blocks 103b/103a/102/107. Again blocks 105 and 103c showed the greatest ChE depression (Figure 2.8).

3.1.3 White-throated Sparrow - Day of Spray

No significant differences (Kruskal - Wallis test*

*Sample variances are heterogenous

T=10.09; d.f. 6, n=7, Prob X>10.09 = 0.1209) were observed between control and spray block samples collected on the day of spray. Once again the lowest sample means were observed in spray blocks 105 and 103c (Figure 2.9).

3.1.4 White-throated Sparrow - Day After Spray

There was a significant difference (Kruskal - Wallis test* T=25.40, d.f. = 6, n = 7, Prob X > 25.40 = 0.0003) observed between sample population means. A multiple range test could not be conducted (unequal sample sizes, see Zar 1974, pg. 157) but the lowest sample means were observed in spray blocks 105 and 103c (Figure 2.9).

*Sample variances are heterogenous

3.2 DIFFERENCES AMONG COLLECTION DAYS - EXTENSIVE BLOCKS

3.2.1 Spray Block 102

Blackpoll Warbler

There was a significant difference (ANOVA $F=5.67$, $d.f. = 2$, $n = 3$, $p < .01$) among control sample and collection day sample means but no significant difference between collection day sample means.

White-throated Sparrow

No significant differences were observed among collection days and control sample means for White-throated Sparrows.

3.2.2 Spray Block 103a

Blackpoll Warbler

A test for significant differences among control and collection day sample means was inconclusive. The test for significant difference was considered

inconclusive if a significant difference was observed in either the Duncan's multiple range test or the Newman-Kuels multiple range test but not both. There was a significant difference between the control sample means and day after spray sample means (Duncan's, Newman-Keuls multiple range test).

White-throated Sparrow

A test for significant differences among control and collection day sample means was inconclusive. The test for significant differences was considered inconclusive if a significant difference was observed in either the Duncan's multiple range test or the Newman-Keuls multiple range test but not both. There was a significant difference between control and day after spray sample means (Duncan's, Newman-Keuls multiple range test).

3.2.3 Spray Block 103b

Blackpoll Warbler

There was a significant difference (ANOVA F=7.34, d.f. = 2, n=3, P < .01) between control samples and collection day samples but no significant difference between collection day samples.

White-throated Sparrow

There was no significant difference between control and collection day samples.

3.2.4 Spray Block 103c

Blackpoll Warbler

There was a significant difference (ANOVA F=19.49, d.f. = 2, n = 3, p < .01) between control and collection day sample means.

White-throated Sparrow

There was a significant difference (ANOVA F=12.16, d.f. = 2, n= 3, $p < .01$) between the means of the control and day of spray samples and the mean of the day after spray samples. There was no significant difference between the mean of the control samples and the mean of the day of spray samples.

3.2.5 Spray Block 105 (First Application)

Blackpoll Warbler

There was a significant difference (ANOVA F=28.02, d.f. 2, n = 3, $p < .01$) between the control sample mean, day of spray sample mean, and day after spray sample mean.

White-throated Sparrow

There was no significant difference between control and day of spray sample means. There was a significant difference between the control sample mean and

the day after spray sample mean (Duncan's, Newman-Keuls multiple range test).

3.2.6 Spray Block 107

Blackpoll Warbler

No Blackpoll Warblers were collected on the day of spray. No significant difference was observed between the control sample mean and the day after spray sample mean.

White-throated Sparrow

No significant difference was observed between control, day of spray and day after spray sample means.

3.3 INTENSIVE BLOCK - CONTROL AND ALL COLLECTION DAYS

3.3.1 Blackpoll Warbler

First Spray Application

There was a significant difference (ANOVA $F=17.18$, $d.f.=3$, $n = 4$, $p < .01$) amongst the control and collection day sample means. The control sample mean was significantly higher than the collection day sample means (Duncan's, Newman-Keuls multiple range test).

Second Spray Application

There was a significant difference (ANOVA $F= 33.71$, $d.f. = 3$, $n = 4$, $p < .01$) amongst the control and collection sample means. The control sample mean was significantly higher than the collection day sample means (Duncan's, Newman - Keuls multiple range test).

3.3.2 Yellow-rumped Warbler

First Spray Application

There was a significant difference (ANOVA $F=19.75$ d.f.$= 3$, $n = 4$, $p < .01$) amongst the control and collection day sample means. The control sample mean was significantly higher than the collection day sample means (Duncan's, Newman-Keuls multiple range test).

Second Spray Application

There was a significant difference (ANOVA $F=16.44$, d.f. $= 3$, $n=4$, $p < .01$) amongst the control and collection day sample means. The control sample mean was significantly higher than the collection day sample means (Duncan's, Newman-Keuls multiple range test).

3.3.3 White-throated Sparrow

First Spray Application

There was a significant difference (ANOVA $F=5.18$, $d.f. = 3$, $n = 4$, $p < .01$) amongst the control and collection day sample means. There was a significant difference between control and day of spray sample means (Duncan's, Newman-Keuls multiple range test).

Second Spray Application

There was a significant difference (ANOVA $F=12.75$, $d.f. = 3$, $n = 4$, $p < .01$) amongst the control and collection day sample means. There was no significant difference between control and day of spray sample (Duncan's, Newman-Keuls multiple range test)

3.4 COMPOSITION OF SONGBIRD SAMPLES

The songbird samples collected for each spray block, collection day and species were analyzed for percent showing brain ChE depression and percent showing a

minimum 50% or life-threatening ChE depression according to the criteria of Zinkl et al. (1980).

The analysis of the Blackpoll Warbler data is shown in Figure 3.1. The analysis of the Yellow-rumped Warbler data is shown in Figure 3.2. The analysis of the White-throated Sparrow data is shown in Figure 3.3.

4.0 DISCUSSION

4.1 1987 RESULTS

A summary of the ChE inhibition observed in the songbirds collected in this study is shown in Table 4.1.

It is important to realize that songbird collections are biased in favour of individual birds that are experiencing a smaller depression of ChE than those having severely depressed ChE activity. If songbird mortality occurred due to severe ChE depression there would not be an opportunity to collect them in this study. Collection of birds by shooting is biased

Figure 3.1 Percentage of Blackpoll Warblers collected showing ChE depression. Numbers above bars represent sample size.

* According to the criterion of Zinkl et al. 1980.

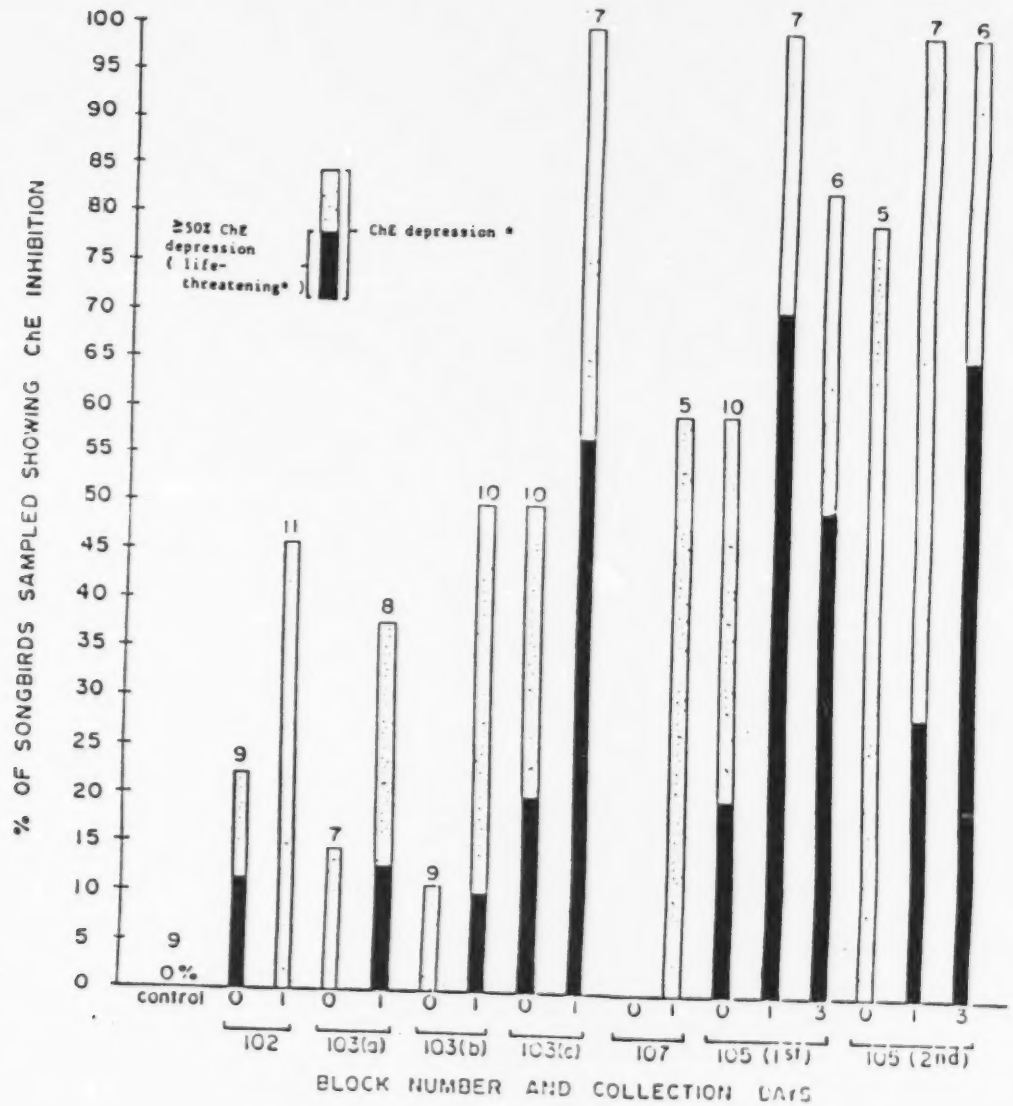

Figure 3.2 Percentage of Yellow-rumped Warblers collected showing ChE depression. Numbers above bars represent sample size.

* According to the criterion of Zinkl et al. 1980.

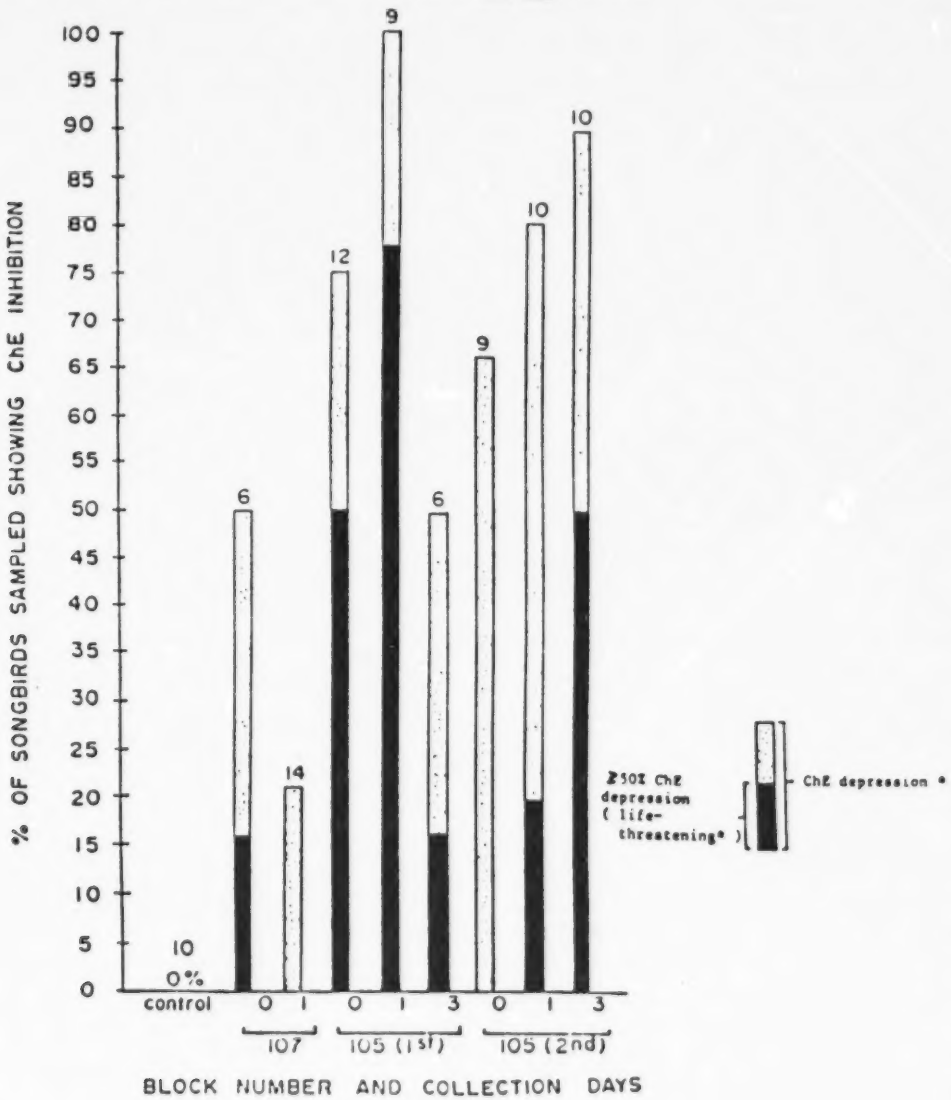

Figure 3.3 Percentage of White-throated Sparrows collected showing ChE depression. Numbers above bars represent sample size.

* According to the criterion of Zinkl et al. 1980.

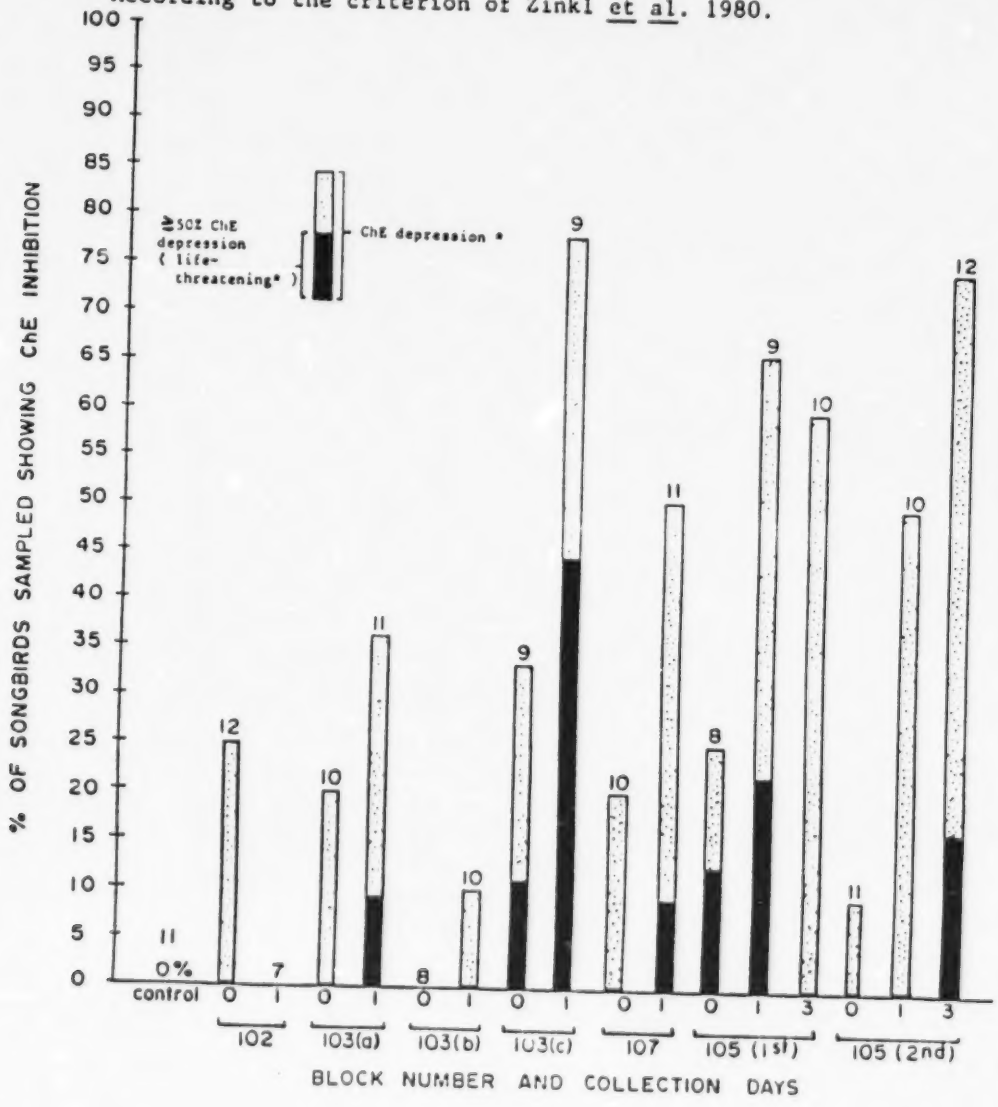

Table 4.1 Number and percentage of songbirds collected in five extensive and one intensive spray blocks showing depression in brain ChE activity and songbirds exhibiting a potential life-threatening depression in brain ChE activity according to Zinkl et al. (1980)

Songbird Species	Total # Collected	Depressed brain ChE Activity	Life-threatening depression of brain ChE activity
	n	n (%)	n (%)
Control			
Blackpoll Warbler	9	(0.0)	(0.0)
Yellow-rumped Warbler	10	(0.0)	(0.0)
White-throated Sparrow	11	(0.0)	(0.0)
Total	30	(0.0)	(0.0)
Post Spray			
Blackpoll Warbler	106	67 (63.2)	25 (23.6)
Yellow-rumped Warbler	76	50 (65.8)	22 (28.4)
White-throated Sparrow	157	54 (34.4)	12 (7.6)
Total	339	171 (50.4)	59 (17.4)

toward those that are singing or active and generally more conspicuous.

It is possible during such collecting methods that any particular songbird may have recently entered the spray block and would not have been exposed to the fenitrothion for as long as resident birds. If this occurred it would serve to underestimate the overall ChE depression observed in the block.

The real possibility of a monitoring study such as this to underestimate the degree of ChE depression by having a potentially biased sample was discussed in Mineau and Peakall (1987) and Fudge, Lane and Associates Limited (1987).

The upper and mid canopy foraging warbler species exhibit a greater brain ChE depression than the ground foraging sparrow and the incidence of ChE depression is greater (Table 4.1, Figure 3.1, 3.2, 3.3). The songbird sampling in the extensive blocks showed mean ChE activity was depressed following exposure to a single fenitrothion spray (Figures 2.8 and 2.9). The songbirds collected the day of first

or second spray application in most spray blocks had depressed brain ChE levels when compared to the controls (Figures 2.8, 2.9, 2.10, 2.11, 2.12).

The songbirds collected the day following the first or second spray application, in most spray blocks, showed a further brain ChE depression from the day of spray songbird samples (Figures 2.8, 2.9, 2.10, 2.11, 2.12). This may be the result of collecting songbirds early on the day of spray application which have not been exposed to a ChE inhibitor for as long as birds collected on subsequent days in the same spray block.

All three songbird species showed a recovery in brain ChE activity on the third day after the first spray application (Figures 2.10, 2.11, 2.12). However, after the second spray application all three songbird species showed an increase in brain ChE depression without any indication of recovery (Figures 2.10, 2.11, 2.12).

In block 105, the percentage of exposed birds decreased from the day after spray to the third day

after the first spray application (Figure 3.1, 3.2, 3.3). However, in block 105 after the second spray application the percentage of exposed birds increased on each successive collection day (Figures 3.1, 3.2, 3.3).

In terms of possible adverse effects of fenitrothion spray on forest songbirds, the lack of any signs of recovery in ChE depression in songbirds collected following the second spray application is noteworthy. Whether the observed lack of recovery in ChE depression in the intensive block second spray is typical in forest spray operations is not known. However, if physiological and behavioural dysfunction are the result of brain ChE depression (see Section 1.0) a prolonged ChE depression following a repeated exposure to fenitrothion may have more severe biological consequences to songbirds than a sample exposure.

The extensive block songbird collections were not sufficient to demonstrate any trend for songbirds recovering from ChE inhibition following the spray application (Figures 3.1, 3.2, 3.3). Generally, a

higher percentage of exposed birds were observed on the day following spray compared to the day of spray.

The occurrence of songbirds receiving a potentially life-threatening depression in ChE activity was not uniformly distributed amongst the six spray blocks. In fact 89.8% of the birds exposed to a potentially life-threatening depression in ChE activity were collected in extensive block 103c and intensive block 105. These results indicate variability in the effect of fenitrothion on songbirds amongst spray blocks in an operational forest spray program.

4.2 COMPARISON WITH 1986 RESULTS

In 1986, 70.9% of the songbirds collected after the spray application were considered to have been exposed to a ChE inhibitor (Fudge, Lane and Associates 1987). In 1987 50.4% of the songbirds collected post-spray exhibited brain ChE depression (Table 4.1).

In 1986, 38.1% of the songbirds collected after the spray application were considered to have been

exposed to a potentially life-threatening amount of ChE inhibitor (Fudge, Lane and Associates 1987). In 1987, this value was 17.4% (Table 4.1).

There appears to be an overall reduction in the percentage of songbirds exhibiting a potentially life-threatening depression in ChE activity after exposure to fenitrothion from the 1986 monitoring study to the results of this study (38.1% in 1986 to 17.4% in 1987). The reduction in the percentage of birds affected is not a result of fenitrothion formulation or application methodology changes.

The results of the 1986 and 1987 monitoring studies reflect the variability in the effect fenitrothion spray application can have on forest songbirds. In 1987, most of the seriously affected songbirds were located in 2 spray blocks.

However, the 1987 monitoring study may more accurately reflect the percentage of songbirds seriously affected in an overall spray program since more spray blocks were monitored in comparison to 1986.

It is considered unacceptable to have 17.4% of songbirds collected exhibiting potentially life-threatening depression in ChE activity following exposure to fenitrothion (D. Busby pers. comm).

4.3 FOLIAGE DEPOSIT ANALYSIS

The results of this study aimed at assessing the deposit of fenitrothion on forest vegetation in the spray blocks is shown in Table 2.1. The results show that the mean deposit of fenitrothion on foliage samples for any block ranged from 0.75 µg/g to 4.90 µg/g fenitrothion. Individual foliage samples collected in all blocks had a range in values from < 0.01 µg/g (or no detectable fenitrothion) to 13.50 µg/g fenitrothion.

There was no correlation between the fenitrothion deposit observed on the foliage and the ChE depression observed in songbirds collected. The foliage deposit results point out that there is not a uniform deposit of fenitrothion across the entire spray block. Areas of high concentrations may be detected by collecting foliage and subsequent analysis.

The variability of fenitrothion deposition in the forest vegetation may account for some of the variation in brain ChE depression observed in the songbirds collected. Evidence suggests that there is a negative correlation between the dose of acetylcholinesterase inhibitors ingested by a bird and the brain ChE activity observed (Mineau and Peakall, 1987; Fleming, 1981; Fleming and Grue, 1981; Fleming and Bradbury, 1981).

Thus the lack of correlation in brain ChE depression and foliage deposit observed in the spray blocks indicate that all songbirds are not exposed to the same amount of fenitrothion.

In this study the majority of the songbirds exhibiting a potentially life-threatening depression in ChE activity at the time of collection were found in spray blocks 103c and 105. However, the fenitrothion residue analysis from balsam fir foliage indicated that a higher deposit of fenitrothion occurred in spray blocks 102 and 103a (Table 2.1).

Foliage deposit studies are important to determine the variation in the amount of fenitrothion occurring at the point of contact with non-target organisms.

4.4 BIOLOGICAL CONSEQUENCES OF ChE DEPRESSION

Depression in brain ChE activity and the corresponding biological consequences is not well known. Researchers have arbitrarily determined that a songbird showing $\geq$ 50% ChE depression could be considered to be in a life-threatening situation (Ludke et al. 1975; Zinkl et al. 1977, 1979, 1980). Obviously birds in this study that exhibited brain ChE depressions $\geq$ 50% of that observed in the control sample were not dead, otherwise they couldn't have been collected for this study.

The biological concern over brain ChE depression in forest songbirds during the nesting season is that temporary ChE depression in adult birds during egg laying, egg incubation or the period of adults feeding nestlings may result in decreased breeding performance.

This effects monitoring study of brain ChE depression focuses on the individual songbird. However, effects monitoring at the songbird population level has been attempted involving field studies of songbird breeding performance in sprayed and unsprayed areas. The problem encountered in effects monitoring at the population level using ChE activity as a measurement of effect, the bird must be sacrificed which prohibits researchers from directly measuring breeding performance of that individual.

Pearce and Busby (1980) reported the results of a study in which White-throated Sparrows were monitored for reproductive parameters and other White-throated Sparrows were collected for brain ChE determination. Both groups were subjected to the same fenitrothion spray. In the study, mean ChE depression in exposed birds was 42% after the first spray and 29% after the second spray (Pearce and Busby, 1980). They found that the reproductive success was severely reduced in birds monitored in the sprayed area. Young fledged from only 11.6% of the eggs laid in nests in sprayed areas compared to young fledgling from 58.3% of eggs

laid in nests from unsprayed areas (Pearce and Busby, 1980).

However, Spray et al. (1987) investigated the effects of fenitrothion spray applications on forest songbirds and reported results indicating little or no effect on songbirds from sprayed plots compared to unsprayed plots. Brain ChE analysis was not performed in conjunction with this study.

Laboratory studies aimed at determining the biological consequences of fenitrothion on birds have been conducted. These studies indicate that most birds exposed to organophosphorus pesticides do recover from depressed ChE levels (Fleming 1981; Fleming and Bradbury, 1981; Fleming and Grue, 1981; Miyamoto 1977). Miyamoto (1977) reported no noticeable differences in terms of egg production, egg weight and quality of eggs of Bobwhite Quail (Colinus virginianus) in the laboratory between control birds and ones fed a diet containing 3 ppm and 10 ppm fenitrothion. However, the mortality of

his fenitrothion fed birds increased over that observed in control birds by 33% (10 ppm fenitrothion feed) and 133% (3 ppm fenitrothion feed) (Miyamoto 1977). Fleming and Bradbury (1981) also report mortality of laboratory test birds having been feed diets containing organophosphorus pesticides. Mortality of birds and other wildlife from operational spray programs has been reviewed and reported in Grue et al. (1983).

Experimental conditions in laboratories create an artificial situation (artificial environment, food and water ad libitum, no predators, etc.) which may not produce results that are useful for predicting what is happening in the field. Direct mortality in field spraying operations is not the prime issue as far as adverse effects on forest songbirds is concerned. Any debilitating effect of sub-lethal organophosphorus pesticides on forest songbirds that may temporarily make the songbirds less fit by negatively affecting nest building, courtship, egg laying, incubation, brood rearing or predator avoidance behaviour will adversely affect the breeding performance of that individual. More studies and research in the field is where the thrust

in wildlife ecotoxicology studies are required rather than in the laboratory.

The biological consequence of brain ChE depression is not resolved. The studies by Pearce and Busby (1980) and Spray et al. (1987) had disparate results and conclusions. However, the study species and methodology, spray application methodology and determination of spray effect were different as to make comparison difficult.

Field studies of operational spray programs are needed to better address the biological consequences of fenitrothion on forest songbirds in Newfoundland.

4.5 DIRECTION OF FUTURE MONITORING

Monitoring studies should assess effects at the population level if an overall evaluation of forest spraying with fenitrothion effects on songbirds is desired. Field studies similar to Pearce and Busby (1980) and Spray et al. (1987) are required in Newfoundland to begin to answer effects monitoring questions at the population level.

Monitoring studies should now focus on the cumulative effect of 2 fenitrothion applications on the same songbird population. The 1987 monitoring study identified the lack of recovery in brain ChE depression in songbirds exposed to fenitrothion spray 4 days apart in the intensive monitoring block.

The monitoring studies conducted in Newfoundland in 1985, 1986 and 1987 are useful in describing the effect of fenitrothion on individual birds. Field studies are required to further develop and understanding of the biological consequences of brain ChE depression.

5.0 CONCLUSIONS

In 1987, the spraying of fenitrothion at 210 g AI/ha resulted in 17.4% of the songbirds collected receiving a potentially life-threatening depression in ChE activity following exposure to fenitrothion, according to the criterion of Zinkl et al. (1980).

Songbird brain ChE activity was depressed on the day of spray when compared to the control samples of the

same species. Generally, songbird brain ChE activity on the day after spray was depressed more than the songbird brain ChE activity observed on the day of spray.

After the first spray application, a trend toward a recovery of brain ChE activity was observed in collections made on the third day following spray. This trend however, was not observed following the second spray application on the same block. Instead the songbird brain ChE activity showed a further depression. The cumulative effect of the second spray application on songbirds previously exposed to fenitrothion is therefore an area of concern. Only one second application on a spray block was monitored and it is not known if the observed trend of continued ChE depression (i.e. no post spray recovery after 3 days) is common with all second spray applications.

The percentage of songbirds showing exposure to a ChE inhibitor increased with time after exposure following the second spray application in the intensive spray block.

The typically mid-canopy and upper canopy foraging warbler species showed higher brain ChE depression than the typically ground foraging sparrow. Whether this is due to the foraging level of the particular species or a response due to differences in bird size or species is not known.

The depression of songbird brain ChE activity was not uniformly distributed in the spray blocks monitored. Spray blocks 103c and 105 had the majority of seriously affected songbirds. Approximately 90% of the songbirds that exhibited a potentially life-threatening depression in ChE activity following exposure to fenitrothion were collected in spray blocks 103c and 105. The adverse effects of fenitrothion on forest songbird brain ChE activity is highly variable amongst the spray blocks monitored.

The deposit study showed that the deposition of fenitrothion in any particular spray block is variable. Also, the deposition of fenitrothion amongst spray blocks is highly variable. The spray blocks that yielded the highest amount of fenitrothion deposition in balsam fir foliage did not

correspondingly exhibit the largest depression in songbird brain ChE activity.

In Newfoundland, from the results of the 1986 and 1987 songbird monitoring studies there is concern over forest songbird safety in operational forest spraying activities using fenitrothion. However, the Forest Pest Management Institute states that field studies have rather conclusively demonstrated that conventional fenitrothion applications do not cause population level impacts on forest songbirds, even with repeated annual treatments (Kingsbury, 1988).

Kingsbury (1988) refers to field studies such as casualty surveys (looking for dead birds), singing bird census techniques and brain ChE studies. These types of studies, however, monitor effects at the individual level (behaviour, ChE depression) rather than effects at the population level (breeding performance).

The adverse effects of fenitrothion on forest songbirds are well documented (see Section 1.0) and

forest managers should continue to monitor the potential adverse effects of fenitrothion on forest songbirds as part of their forest management programs. The results of the 1986 and 1987 songbird monitoring studies conducted in Newfoundland appear not to support the conclusions of the Forest Pest Management Institute.

6.0 RECOMMENDATIONS

Based on the results of this study, there is concern about songbird safety and the ability of songbirds to recover from a repeated exposure to fenitrothion under operational spray conditions. Future monitoring studies should address this concern. If fenitrothion is going to be applied in the future at 210 g AI/ha on forests in Newfoundland and Labrador monitoring studies on songbirds should continue.

Monitoring studies should be designed to determine the adverse effect of fenitrothion on forest songbirds, the variability of the effect on songbirds amongst spray blocks and the time required for songbird populations to recover after exposure to

first and second spray applications. Field studies should be designed to assess the biological consequences of brain ChE depression in forest songbirds. This will lead to a better understanding of environmental effects at the population level.

Provincial and federal environment agencies should address the issue of developing acceptable standards (such as acceptable percentage of songbirds showing brain ChE depression or $\geq$ 50% brain ChE depression) concerning the adverse effects of fenitrothion on songbirds during operational forest protection programs. Once standards are developed DFRL will be better able to protect forests in an environmentally acceptable manner.

Foliage deposit studies should focus on collecting more samples throughout each block to more accurately determine an average deposition value for each block. Foliage samples should also be collected on the day of each spray and for several days after each spray in any particular block. This may help determine the persistence of fenitrothion and how long it is effective on songbirds. It will also indicate if

fenitrothion accumulates after the second spray to better understand second exposure effects of fenitrothion on forest songbirds.

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8.0 PERSONAL COMMUNICATIONS

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APPENDIX A

Brain ChE Activity in Songbirds Collected During
1987 Fenitrothion Monitoring Study

(ChE activity expressed as micromoles
hydrolyzed/minute/g brain, wet weight)

CONTROL SAMPLES

White-throated Sparrow	Yellow-rumped Warbler	Blackpoll Warbler
32.65	41.14	29.32
28.41	29.78	27.51
27.96	27.96	32.16
25.76	28.86	27.96
28.86	30.72	27.96
28.41	27.51	27.96
27.51	29.78	34.14
30.25	32.16	33.14
30.72	30.72	31.68
28.86	27.07	
29.78		

Spray Block 102

White-throated Sparrow	Day of Spray	Blackpoll Warbler
30.72		27.51
29.32		25.33
31.68		26.19
26.19		31.68
29.78		28.86
28.86		29.32
25.76		27.96
20.43		13.85
30.25		20.43
19.66		
29.78		
21.62		
	Day after Spray	
31.68		21.62
28.86		30.25
32.65		28.86
24.06		24.48
29.32		24.06
28.86		22.02
30.72		24.90
		20.83
		22.42
		24.48
		27.07

Spray Block 103a

White-throated Sparrow	Day of Spray	Blackpoll Warbler
16.68		24.90
27.96		28.41
27.51		27.96
31.20		20.83
27.51		30.25
28.86		29.32
28.86		24.48
16.68		
27.07		
31.20		
	Day After Spray	
25.33		31.20
26.63		26.63
27.51		27.07
8.92		20.43
27.07		11.82
14.54		26.19
21.22		15.60
18.90		30.25
29.78		
16.68		
30.72		

Spray Block 103b

White-throated Sparrow	Day of Spray	Blackpoll Warbler
32.65		24.90
27.07		26.63
29.78		28.41
32.16		25.76
27.07		26.63
27.07		27.96
32.65		25.33
25.76		29.32
		18.52
	Day After Spray	
36.20		18.90
24.90		22.42
28.41		26.19
33.14		25.33
27.96		24.90
24.90		28.41
20.83		11.82
23.65		29.78
31.20		24.06
26.63		23.23

Spray Block 103c

White-throated Sparrow	Day of Spray	Blackpoll Warbler
25.76		21.22
26.19		26.63
26.63		18.15
18.15		9.24
27.96		11.82
13.85		26.63
28.41		26.19
30.72		
15.24		
	Day After Spray	
15.24		17.78
29.78		7.68
17.04		20.83
5.00		19.66
17.41		13.17
14.19		9.24
10.84		10.52
27.51		
7.68		

Spray Block 105 (first application)

White-throated Sparrow	Yellow-rumped Warbler	Blackpoll Warbler
	Day of Spray	
34.65	8.92	24.90
18.15	27.51	30.72
27.96	23.23	25.33
24.48	26.63	19.66
27.96	21.62	13.17
25.76	10.52	20.43
11.82	10.84	17.04
26.19	14.19	22.42
	9.24	12.83
	18.90	28.86
	13.51	
	17.04	
	First Day After Spray	
14.19	14.19	9.24
11.49	9.55	19.28
24.90	13.51	17.41
21.22	9.24	12.83
21.62	11.49	8.61
22.83	12.16	12.49
14.89	12.83	10.19
27.96	19.66	
26.19	15.60	

Spray Block 105 (first application) (cont'd)

White-throated Sparrow	Yellow-rumped Warbler	Blackpoll Warbler
	Third Day After Spray	
26.63	26.19	22.42
27.07	8.30	8.92
22.83	21.22	15.95
22.22	21.22	26.19
18.15	24.06	8.61
16.68	23.65	10.19
14.89		
24.06		
22.02		
31.68		

Spray Block 105 (second application)

White-throated Sparrow	Yellow-rumped Warbler	Blackpoll Warbler
	Day of Spray	
28.41	22.02	15.24
24.90	26.63	24.90
27.51	24.06	15.95
28.86	20.05	22.42
29.32	21.22	19.28
33.14	19.66	
29.32	19.28	
17.41	20.83	
27.07	22.83	
26.63		
28.41		
	First Day After Spray	
18.15	16.31	22.02
21.22	17.78	8.61
30.25	18.90	16.68
27.96	22.42	17.04
19.28	27.07	16.68
26.19	23.65	17.04
16.68	13.17	13.51
25.33	6.47	
26.19	19.28	
	15.60	

Spray Block 105 (second application) (cont'd)

White-throated Sparrow	Yellow-rumped Warbler	Blackpoll Warbler
	Third Day After Spray	
14.19	14.19	9.55
24.90	20.83	12.16
25.33	12.49	19.28
14.54	10.84	16.31
19.66	13.51	14.19
27.96	17.41	10.52
21.62	27.96	
19.66	14.51	
18.90	20.83	
11.82	23.65	
20.43		
17.41		

Spray Block 107

White-throated Sparrow	Yellow-rumped Warbler	Blackpoll Warbler
	Day of Spray	
24.48	11.49	
29.78	18.52	
26.63	24.48	
20.83	22.83	
26.63	26.19	
30.25	19.28	
27.51		
27.96		
21.62		
27.96		
	Day After Spray	
28.41	31.68	24.06
12.83	21.22	21.22
29.32	30.72	29.32
14.54	29.78	21.22
32.16	27.51	33.64
30.72	20.43	
22.42	28.86	
30.25	34.65	
32.65	18.15	
30.72	33.14	
28.86	29.32	
	30.72	
	31.20	
	29.78	

APPENDIX B

METHODOLOGY FOR THE DETERMINATION OF FENITROTHION DEPOSIT IN FOLIAGE

DETERMINATION OF FENITROTHION DEPOSIT IN FOLIAGE

This method is based on Reynolds, L.M., Determination of Fenitrothion and Matacil Residues in Newfoundland Samples, ORF, 1977.

Materials and Reagents

Fenitrothion analytical standard

Solvents - all distilled in glass

Florisil - 60/100 mesh, heated at 600°C overnight, then decativated with 2% water

Florisil columns - 2.5 x 30 cm, glass frit with ground glass stopcock

Buchner filter funnels

Waring commercial blender

Porcelain mortar and pestle

Buchi rotary evaporator

Assorted lab glassware, solvent rinsed

Gas Chromatography Equipment and Operating Conditions

Varian 3700 GC

Column - 30 m DB-5 capillary

Temperature program - initial 85°C for 2 min., then

15 C/min. to 230°C final, and 20 min. at final temperature

Director - electron capture

Carrier glass - column nitrogen 2.7 mL/min. (18 psi), Hydrogen 5.8 mL/min. (27 psi), makeup 35 mL/min., air 178 mL/min., purge 12 mL/min.

Intergrator - Hewlett-Packard 3380A

Method

1. More than ten grams of sample was placed in a mortar and frozen with liquid nitrogen. The frozen sample was ground with a pestle and allowed to thaw. Ten (+/- 0.01) grams of sample was placed in a blender with 100 mL ethyl acetate and blended for 30 s at full power.

2. The blended sample was poured into a Buchner funnel and the blender rinsed with two portions of ethyl acetate to make up 250 mL total volumn. The solvent was pulled through with gentle suction.

3. The solvent was transferred to a round-bottomed

flask, taken to dryness and taken up in 5 mL of 35% methylene chloride in hexane.

4. Columns were made up by wet packing 12 cm florisil in hexane into the 2.5 cm glass columns, and adding 1 cm sodium sulfate to the top.

5. The sample was transferred to the top of the column and eluted with 250 mL 35% methylene chloride in hexane.

6. The eluate was concentrated to almost-dryness on a rotary evaporator and taken up in 2.0 mL wthyl acetate for GC analysis.

7. Injections of 0.8 uL were made in duplicate, with daily standards. Determinations were made by comparison of electronically integrated peak areas.